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(54) **NUCLEIC ACID CONSTRUCTS METHODS
FOR ALTERING PLANT FIBER LENGTH
AND/OR PLANT HEIGHT**

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(2013.01); **C12N 15/8226** (2013.01); **C12N**
15/8241 (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

Nucleic acid constructs and methods are disclosed for modifying fiber length, plant height, and/or plant biomass in plant tissues. Plants are genetically engineered with constructs encoding an *Arabidopsis thaliana* wall-associated kinase gene, which alters fiber length and/or plant height when over-expressed under the control of a cambium/xylem preferred promoter. Plant transformants harboring a wall-associated kinase gene show increased fiber length, a trait that is thought to improve woody trees for pulping and papermaking.

19 Claims, 5 Drawing Sheets

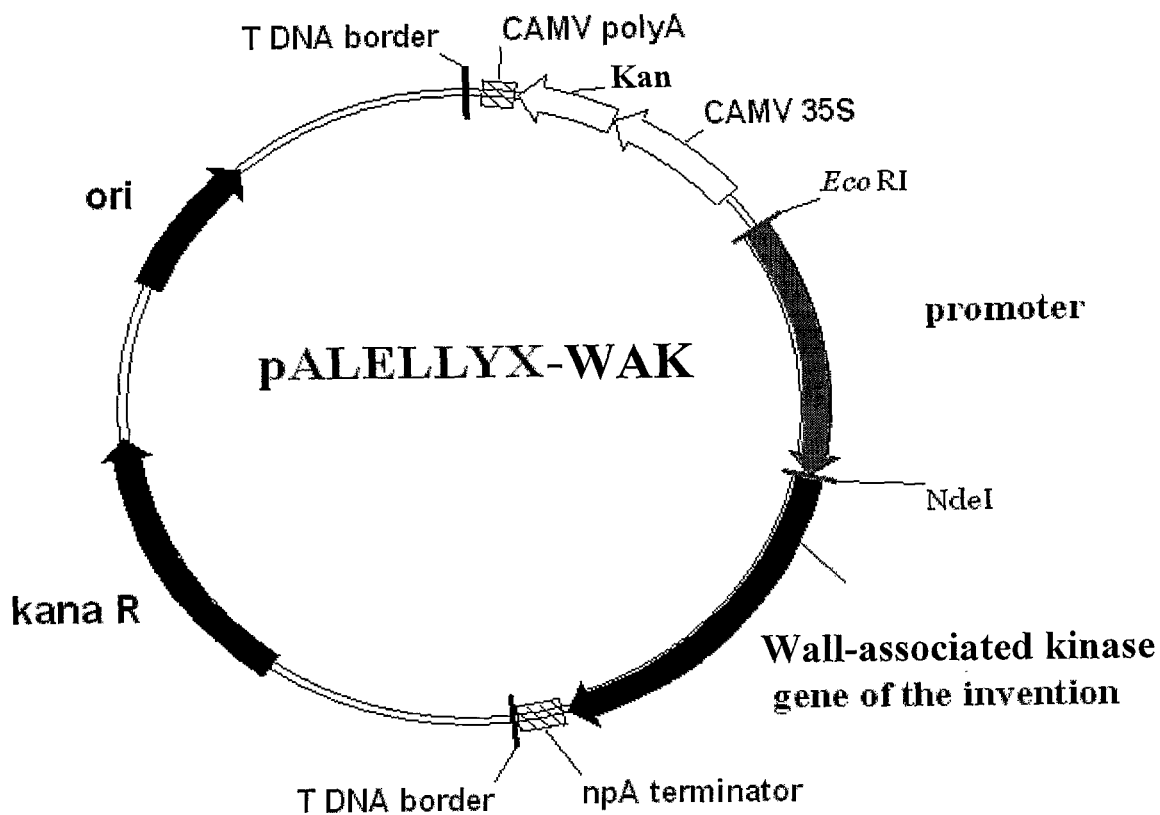
FIG. 1

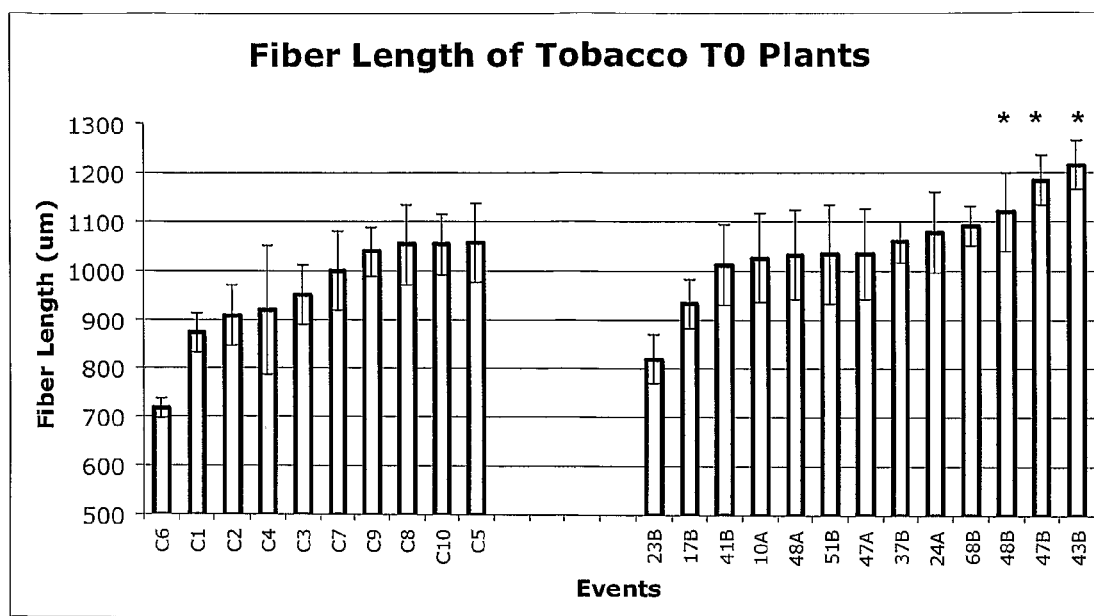
FIG. 2

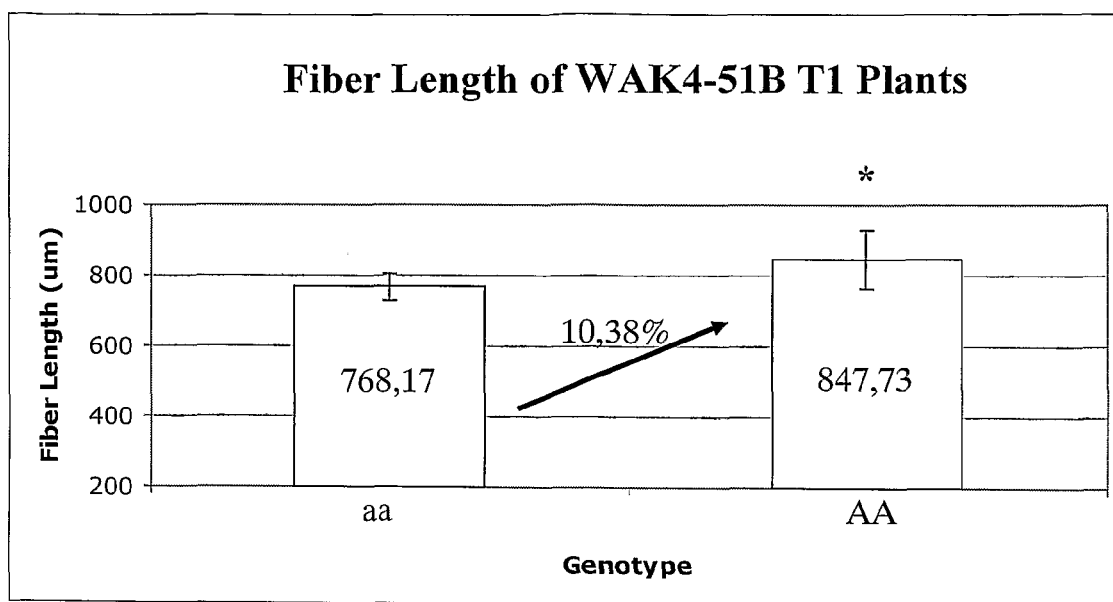
FIG. 3

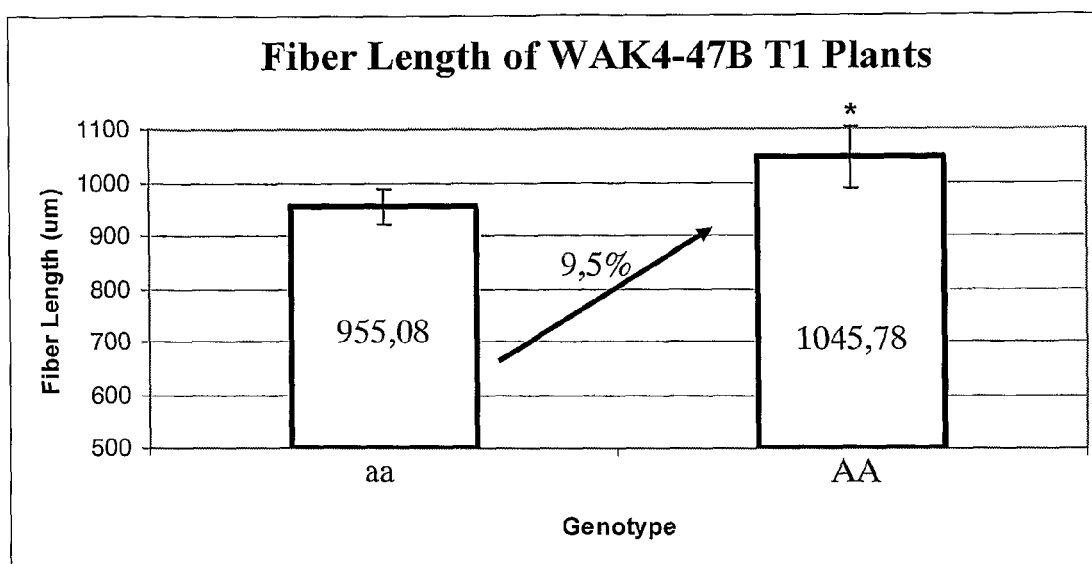
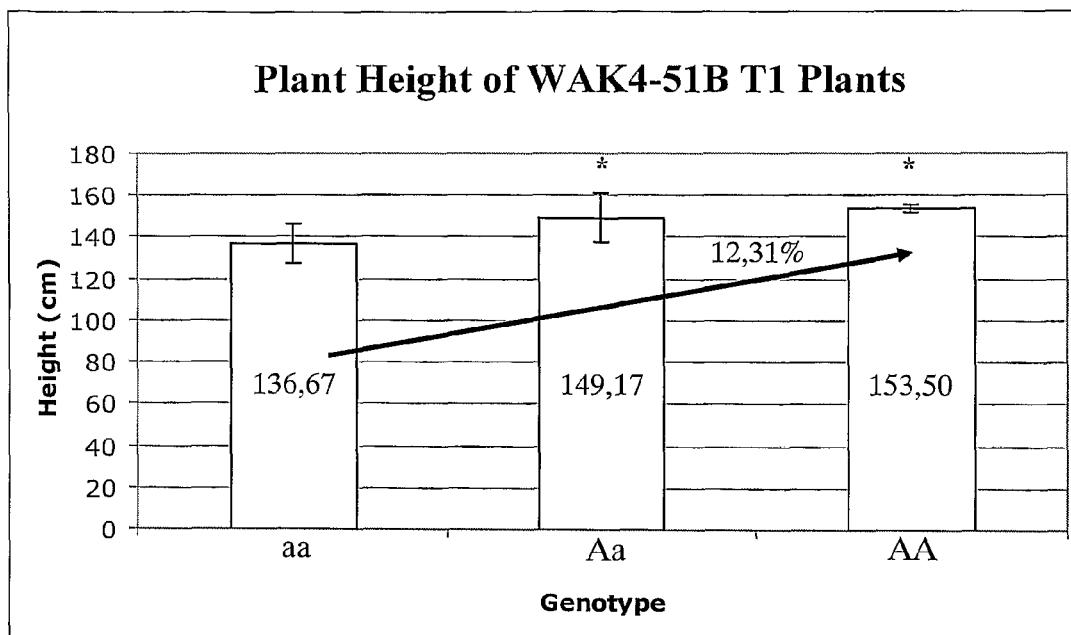
FIG. 4

FIG. 5

NUCLEIC ACID CONSTRUCTS METHODS FOR ALTERING PLANT FIBER LENGTH AND/OR PLANT HEIGHT

CROSS-REFERENCE TO RELATED APPLICATION

This application is the National Stage of International Application No. PCT/BR07/00357, filed Dec. 20, 2007, which claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional Application No. 60/871,048, filed Dec. 20, 2006, the disclosure of which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to the fields of molecular biology and alteration of gene expression in transformed plants. More specifically, this invention relates to the modification of fiber length and/or plant height in plants of industrial interest by regulation of expression of genes encoding wall-associated kinases (WAKs).

BACKGROUND OF THE INVENTION

The increasing demand for wood products and wood derived products constitutes a problem of global proportion. It is estimated that the maximum sustainable rate of harvesting from the world's forests has already been reached. Thus, there is an imminent need for more woody plants, as well as a need for developing methods for increasing the agronomic properties of forestry plants, such as enhanced plant height, enhanced biomass production, and longer xylem fiber length. For example, fiber uniformity and strength are common requirements for most industrial uses. In pulp manufacture, strength characteristics are determined in part by fiber length. Long fibers are ideal for strong paper production, pulp yield increase and decrease in alkali consumption, due to their strength and bonding properties.

As an illustrative example of the importance of woody plants, one can mention *Eucalyptus* trees, which represent the largest sources of fibers used globally in the paper industry. Bamber, 1985, *Appita* 38: 210-216). There are an estimated ten to fifteen million hectares of land planted with *Eucalyptus*. Verhaegen and Plomion, 1996, *Genome* 39: 1051-1061. The major advantage of the *Eucalyptus* tree is its very high growth rate and ability to grow in a wide range of conditions, both tropical and temperate. The *Eucalyptus* fibers have one disadvantage, however, compared to fibers from other sources, such as pine, which is their significantly shorter length. Thus, papers that are made from *Eucalyptus* pulp are often weak and usually require reinforcement with longer fibers from other sources increasing the production costs.

Fiber length is controlled by endogenous regulation of cell elongation, a process which results from the interaction between internal turgor pressure and the mechanical strength of the cell wall, but its mechanism and genes involved have not been yet totally discerned.

Xylem fiber cells develop from already much-elongated fusiform initials located within the vascular cambium. They increase in diameter by extension of their radial walls, and, in addition, developing fiber cells elongate by intrusive tip growth, which results in up to a severalfold increase in cell length. Gray-Mitsumune et al., 2004, *Plant Physiol.* 135: 1552-1564.

In tip-growing cells, expansion occurs over a small area of the cell surface, which results in tubular, elongated cells. For

example, poplar fibers elongate intrusively in the radial-expansion zone in the xylem, reaching 150% of their initial cell length at the average when fully differentiated. Hussey et al., 2006, *Annu. Rev. Plant Biol.* 57: 109-125; Mellerowicz et al., 2001, *Plant Mol. Biol.* 47: 239-274.

The rapid expansion of fiber cells may be achieved by concerted action of pushing against the cell wall exerted by turgor and loosening of the cell wall. In cotton fibers, the phase of cell elongation follows a significant rise of turgor, resulted from the observed accumulation of malate, sugars, and K⁺, the major osmoticum, hence the influx of water and the generation of high turgor in the fiber cells. Ruan et al., 2004, *Plant Physiol.* 136: 4104-4113.

Vacuolar invertases can play an important role in turgor maintenance and cell wall expansion. Recent work in *Arabidopsis thaliana* has shown that a wall-associated kinase (WAK) can regulate a vacuolar invertase thus establishing a cross-compartmental link between WAK and vacuolar invertase(s). Kohorn et al., 2006, *Plant J.* 46: 307-316.

In *Arabidopsis* WAKs are encoded by five tightly linked and highly similar genes, and are expressed in leaves, meristems, and cells undergoing expansion. Wagner and Kohorn, 2001, *Plant Cell* 13: 303-318.

Mutant seedlings of *Arabidopsis thaliana* presenting a T-DNA insertion in the WAK2 gene were significantly shorter than wild-type plants, with the roots more affected than the hypocotyls. Kohorn et al., 2006, *Plant J.* 46: 307-316.

These mutant plants showed a reduced vacuolar invertase activity by 62%, and the authors proposed that WAK2 regulates the transcription of vacuolar invertase as one constituent of a mechanism modulating solute concentrations and turgor regulation, thus providing a possible mechanism for WAK to regulate cell expansion.

The expression of an inducible antisense WAK2 in *Arabidopsis* led to a 50% reduction in WAK protein levels, with a subsequent loss of cell elongation, and hence dwarf plants. Similar results have been reported when an antisense WAK4 gene was used to reduce total WAK protein levels. Wagner and Kohorn, 2001, *Plant Cell* 13: 303-318; Lally et al., 2001, *Plant Cell* 13: 1317-1331.

It is also known that the wall-associated kinases contain extracellular domains that can be linked to pectin molecules of the cell wall, span the plasma membrane and have a cytoplasmic serine/threonine kinase domain. He et al., 1999, *Plant Mol. Biol.* 39: 1189-1196.

When fibers undergo significant elongation at both ends (intrusive tip growth), the properties of the middle lamella limit this type of cell growth. Middle lamellae of developing wood cells are rich in pectins, and intrusive tip growth requires the dissolution of the middle lamella. See Berthold et al., WO 2006/068603.

By their pectin attachment, it is possible that WAKs may sense a change in the cell wall environment, thus providing a molecular mechanism linking cell wall sensing to regulation of solute metabolism, which in turn is known to be involved in turgor maintenance and cell expansion in growing cells. Such information could be invaluable to adjustment of cell expansion or turgor. Huang et al., 2007, *Functional Plant Biology*, 34: 499-507.

Fiber characteristics are controlled by a complex set of genetic factors and are not easily amenable to classical breeding methods. Through traditional forest tree breeding it is possible to achieve some modification of fiber characteristics. For example, interspecific triploid hybrids of poplar have been developed which have longer fibers than the parental species. Aziz et al., 1996, Wood and pulp properties of aspen and its hybrids. *TAPPI Proc. Pulping Conference*. p. 437-443.

Yet, considering the disadvantage of traditional forest tree breeding, such as the slow progress due to their long generation periods and the difficulty of producing a plant with a desirable trait, the developments in gene technology can reduce significantly the time required to produce a new variety of plant and allow closer targeting of traits considered desirable by the forest and pulp industries in specific trees species.

SUMMARY OF THE INVENTION

In one aspect, the invention provides a nucleic acid construct comprising a WAK polynucleotide sequence operably linked to a xylem-preferred promoter that causes overexpression of said WAK polynucleotide sequence. In an embodiment, the xylem-preferred promoter is selected from the group consisting of TUB gene promoter, SuSy gene promoter, COMT gene promoter and C4H gene promoter. In another embodiment, a transgenic plant comprises the nucleic acid construct and the plant has an increase in fiber length and/or height compared to a non-transgenic plant of the same species. In further embodiments the plant is a dicotyledon, monocotyledon, gymnosperm, or hardwood tree. The invention further contemplates the progeny of the transgenic plant, as well as wood pulp and wood fiber produced from the transgenic plant.

In another aspect, the invention provides a method for increasing fiber length and/or plant height, comprising: (a) introducing into a plant cell a nucleic acid construct comprising a WAK polynucleotide sequence operably linked to a xylem-preferred promoter that causes overexpression of said WAK polynucleotide sequence; (b) culturing said plant cell under conditions that promote growth of a plant; and (c) selecting a transgenic plant that has increased fiber length and/or plant height compared to a non-transgenic plant of the same species.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 schematically illustrates the plant expression plasmidial vector pALELLYX-WAK of the invention comprising a cambium/xylem preferred promoter driving the expression of a wall-associated kinase nucleotide sequence of the invention.

FIG. 2 shows the fiber length of several transgenic lines transformed with the plant expression plasmidial vector pALELLYX-WAK of the invention and respective control non-transgenic plants. Asterisk denotes statistically significant higher mean fiber length values ($P < 0.05$, t-test).

FIG. 3 shows the fiber length of two genotypes of a T1 transgenic plant (line 51B) transformed with the plant expression plasmidial vector pALELLYX-WAK of the invention. Asterisk denotes statistically significant higher mean fiber length values ($P < 0.05$, t-test).

FIG. 4 shows the fiber length of two genotypes of a T1 transgenic plant (line 47B) transformed with the plant expression plasmidial vector pALELLYX-WAK of the invention. Asterisk denotes statistically significant higher mean fiber length values ($P < 0.05$, t-test).

FIG. 5 shows the plant height of the three genotypes of a T1 transgenic line (line 51B) transformed with the plant expression plasmidial vector pALELLYX-WAK of the invention. Asterisk denotes statistically significant higher mean plant height values ($P < 0.05$, t-test).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to processes for genetic manipulation of fiber length in plants and/or an increase in plant height.

The plant cell wall is a strong fibrillar network that gives each cell its stable shape. To enlarge, cells selectively loose this network, enabling it to yield to the expansive forces generated by cell turgor pressure. As a cell expands, there is increased need for a compensatory adjustment in turgor, which is dependent on cell solute metabolism.

A wall-associated kinase (WAK) may sense cell wall expansion by its attachment to pectin, thereby providing a mechanism for transducing these signals to systems regulating solute changes, as outlined above. The previous work on WAKs, however, did not presage that the overexpression of a WAK gene in plant, in a tissue-specific manner, results in significant changes in fiber length, as well as significant changes in plant height. The result opens the way to modifying traits that are extremely important for the plant fiber, forest, pulp, and paper industries.

According to an aspect of the present invention, therefore, a method is provided for modifying the fiber length in plant tissues, such as fiber cells of woody angiosperm xylem, tracheid cells of gymnosperm xylem, and fiber cells of cotton seeds, by controlling the activity of a wall-associated kinase. Pursuant to this aspect of the invention, plant cells or whole plants are genetically engineered with a wall-associated kinase coding sequence, which, when expressed in xylary fiber cells of angiosperms, xylary tracheids of gymnosperms, or fiber cells of cotton seeds, causes an increase in cell length.

All technical terms used herein are terms commonly used in biochemistry, molecular biology and agriculture, and can be understood by one of ordinary skill in the art to which this invention belongs. Those technical terms can be found in: MOLECULAR CLONING: A LABORATORY MANUAL, 3rd ed., vol. 1-3, ed. Sambrook and Russel, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, ed. Ausubel et al., Greene Publishing Associates and Wiley-Interscience, New York, 1988 (with periodic updates); SHORT PROTOCOLS IN MOLECULAR BIOLOGY: A COMPENDIUM OF METHODS FROM CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, 5th ed., vol. 1-2, ed. Ausubel et al., John Wiley & Sons, Inc., 2002; GENOME ANALYSIS: A LABORATORY MANUAL, vol. 1-2, ed. Green et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1997. Methodology involving plant biology techniques is described herein and is described in detail in treatises such as METHODS IN PLANT MOLECULAR BIOLOGY: A LABORATORY COURSE MANUAL, ed. Maliga et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1995. Various techniques using PCR are described, e.g., in Innis et al., PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS, Academic Press, San Diego, 1990 and in Dieffenbach and Dveksler, PCR PRIMER: A LABORATORY MANUAL, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2003. PCR-primer pairs can be derived from known sequences by known techniques such as using computer programs intended for that purpose, e.g., Primer, Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass. Methods for chemical synthesis of nucleic acids are discussed, for example, in Beaucuecci and Caruthers, 1981, *Tetra. Letts.* 22: 1859-1862, and Matteucci and Caruthers, 1981, *J. Am. Chem. Soc.* 103: 3185.

Restriction enzyme digestions, phosphorylations, ligations and transformations were done as described in Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed. (1989), Cold Spring Harbor Laboratory Press. All reagents

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and materials used for the growth and maintenance of bacterial cells were obtained from Aldrich Chemicals (Milwaukee, Wis.), DIFCO Laboratories (Detroit, Mich.), Invitrogen (Gaithersburg, Md.), or Sigma Chemical Company (St. Louis, Mo.) unless otherwise specified.

The terms “encoding” and “coding” refer to the process by which a gene, through the mechanisms of transcription and translation, provides information to a cell from which a series of amino acids can be assembled into a specific amino acid sequence to produce an active enzyme. Because of the degeneracy of the genetic code, certain base changes in DNA sequence do not change the amino acid sequence of a protein. It is therefore understood that modifications in the DNA sequence encoding wall-associated kinase which do not substantially affect the functional properties of the protein are contemplated.

In this description, “expression” denotes the production of the protein product encoded by a gene. Alternatively or additionally, “expression” denotes the combination of intracellular processes, including transcription and translation, undergone by a coding DNA molecule such as a structural gene to produce a polypeptide. “Overexpression” refers to the expression of a particular gene sequence in which the production of mRNA or polypeptide in a transgenic organism exceeds the levels of production in non-transgenic organism.

The term “heterologous nucleic acid” refers to a nucleic acid, DNA or RNA, which has been introduced into a cell (or the cell’s ancestor) through the efforts of humans. Such exogenous nucleic acid may be a copy of a sequence which is naturally found in the cell into which it was introduced, or fragments thereof.

In contrast, the term “endogenous nucleic acid” refers to a nucleic acid, gene, polynucleotide, DNA, RNA, mRNA, or cDNA molecule that is present in a plant or organism that is to be genetically engineered. An endogenous sequence is “native” to, i.e., indigenous to, the plant or organism that is to be genetically engineered.

The term “homologous sequences” refers to polynucleotide or polypeptide sequences that are similar due to common ancestry and sequence conservation.

The term “functional homolog” refers to a polynucleotide or polypeptide sequences that are similar due to common ancestry and sequence conservation and have identical or similar function at the catalytic, cellular, or organismal levels. Wall-Associated Kinase Sequences

In this description, the term “wall-associated kinase polynucleotide sequence” denotes any nucleic acid, gene, polynucleotide, DNA, RNA, mRNA, or cDNA molecule that encodes a wall-associated kinase polypeptide whose overexpression alters fiber length and/or plant height. The DNA or RNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also called the anti-sense strand. Illustrative of this category are polynucleotide molecules that comprise SEQ ID NOs: 1, 3, 5, 7 and 9, identified from *Arabidopsis thaliana* and that can be employed to enhance fiber length and/or plant height.

A wall-associated kinase polynucleotide sequence suitable for the present invention may be identified from a myriad of organisms characterized by the presence of a WAK gene. Although the aforementioned nucleotide sequences are disclosed herein, they are not to be taken as limitations on the present invention. Thus, a WAK sequence can be identified and functionally annotated by sequence comparison. The skilled person can readily identify a functionally related WAK sequence in a suitable database, such as GenBank, using publicly available sequence-analysis programs and

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parameters. Alternatively, screening cDNA libraries or genomic libraries employing suitable hybridization probes or primers based on DNA or protein sequences disclosed herein should lead to the identification of functionally related WAK sequences (functional homolog). It is appreciated in the field as well that sequences with reduced levels of identity also can be isolated with the aid of degenerate oligonucleotides and PCR-based methodology. While the polynucleotides of the inventions are isolated from *Arabidopsis thaliana*, functional homologs from other plants can be employed to produce plants with enhanced fiber length and/or plant height. Examples of plant species from which WAK genes may be isolated include dicotyledons, such as Cucurbitaceae, Solanaceae, Brassicaceae, Papilionaceae such as alfalfa and *Vigna unguiculata*, Malvaceae, Asteraceae, Malpighiaceae such as *Populus*, Myrtaceae such as *Eucalyptus*, and monocotyledons, such as gramineae, including rice, wheat, sugarcane, barley, and corn.

In this description, the terms “wall-associated kinase polynucleotide sequence,” “WAK polynucleotide sequence” and “WAK DNA sequence” also refer to any nucleic acid molecule with a nucleotide sequence capable of hybridizing under stringent conditions with any of the sequences disclosed herein, and coding for a polypeptide with WAK activity equivalent to the proteins having amino acid sequences disclosed herein under SEQ ID NOs: 2, 4, 6, 8, or 10. The terms also include sequences which cross-hybridize with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7 or SEQ ID NO: 9, preferably having at least 65% homology or identity with one or more of SEQ ID NO: 1, 3, 5, 7 or 9. The nucleotide sequences of the invention may encode a protein which is homologous to the predicted gene product disclosed herein under any of SEQ ID NOs: 2, 4, 6, 8, or 10. Further, the nucleotide sequences of the invention include those sequences that encode a WAK polypeptide having an amino acid sequence which has at least 55%, preferably at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably at least 90% and most preferably at least 95% sequence identity to an amino acid sequence disclosed herein under any of SEQ ID NOs: 2, 4, 6, 8 and 10. The degeneracy of the genetic code enables major variations in the nucleotide sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein.

The phrase “stringent conditions” here connotes parameters with which the art is familiar. Single-stranded polynucleotides hybridize when they associate based on a variety of well-characterized physicochemical forces, such as hydrogen bonding, solvent exclusion, and base stacking. The stringency of a hybridization reflects the degree of sequence identity of the nucleic acids involved, such that the higher the stringency, the more similar are the two polynucleotide strands. Stringency is influenced by a variety of factors, including temperature, salt concentration and composition, organic and non-organic additives, solvents, etc. present in both the hybridization and wash solutions and incubations (and number). One with ordinary skill in the art can readily select such conditions by varying the temperature during the hybridization reaction and washing process, the salt concentration during the hybridization reaction and washing process, and so forth.

For hybridization of complementary nucleic acids which have more than 100 complementary residues, on a filter in a Southern or Northern blot, “stringent” hybridization conditions are exemplified by a temperature that is about 5° C. to 20° C. lower than the thermal melting point (T_m) for the specific sequence, at a defined ionic strength and pH. The T_m is the temperature, under defined ionic strength and pH, at

which 50% of the target sequence hybridizes to a perfectly matched probe. Nucleic acid molecules that hybridize under stringent conditions typically will hybridize to a probe based on either the entire cDNA or selected portions. More preferably, “stringent conditions” here refers to parameters with which the art is familiar, such as hybridization in 3.5×SSC, 1×Denhardt’s solution, 25 mM sodium phosphate buffer (pH 7.0), 0.5% SDS, and 2 mM EDTA for 18 hours at 65° C., followed by four washes of the filter, at 65° C. for 20 minutes, in 2×SSC and 0.1% SDS, and a final wash for up to 20 minutes in 0.5×SSC and 0.1% SDS or 0.3×SSC and 0.1% SDS for greater stringency, and 0.1×SSC and 0.1% SDS for even greater stringency. Other conditions may be substituted, as long as the degree of stringency is equal to that provided herein, using a 0.5×SSC final wash. For identification of less closely related homologues washes can be performed at a lower temperature, e.g., 50° C. In general, stringency is increased by raising the wash temperature and/or decreasing the concentration of SSC.

Additionally, the category of suitable wall-associated kinase sequences includes a nucleic acid molecule comprised of a variant of SEQ ID NOs: 1 or 3 or 5 or 7 or 9 with one or more bases deleted, substituted, inserted, or added, which variant codes for a polypeptide when overexpressed results in alteration in fiber length and/or plant height. The “base sequences with one or more bases deleted, substituted, inserted, or added” referred to here are widely known by those having ordinary skill in the art to retain physiological activity even when the amino acid sequence of a protein generally having that physiological activity has one or more amino acids substituted, deleted, inserted, or added. For example, the poly A tail or 5' or 3' end nontranslation regions may be deleted, and bases may be deleted to the extent that amino acids are deleted. Bases may also be substituted, as long as no frame shift results. Bases also may be “added” to the extent that amino acids are added. It is essential, however, that any such modification does not result in the loss of physiological activity. A modified DNA in this context can be obtained by modifying the DNA base sequences of the invention so that amino acids at specific sites are substituted, deleted, inserted, or added by site-specific mutagenesis, for example. Zoller & Smith, 1982, *Nucleic Acid Res.* 10: 6487-6500. Accordingly, the term “variant” is a nucleotide or amino acid sequence that deviates from the standard, or given, nucleotide or amino acid sequence of a particular gene or protein. The variant may have “conservative” changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. A variant may have “nonconservative” changes, e.g., replacement of a glycine with a tryptophan. Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted may be found using computer programs well known in the art such as Vector NTI Suite (InforMax, MD) software. “Variant” may also refer to a “shuffled gene,” as described, for example, in U.S. Pat. Nos. 6,506,603, 6,132,970, 6,165,793 and 6,117,679.

A further way of obtaining a WAIS DNA sequence is to synthesize it ab initio from the appropriate bases, for example, by using the appropriate cDNA sequence as a template.

Nucleic Acid Constructs

The present invention includes recombinant constructs comprising one or more of the nucleic acid sequences herein. The constructs typically comprise a vector, such as a plasmid, a cosmid, a phage, a virus (e.g., a plant virus), a bacterial artificial chromosome (BAC), a yeast artificial chromosome

(YAC), or the like, into which a nucleic acid sequence has been inserted, in a forward or reverse orientation. Large numbers of suitable vectors are known and commercially available and need not be reiterated here.

Recombinant nucleic acid constructs may be made using standard techniques. For example, a nucleotide sequence for transcription may be obtained by treating a vector containing said sequence with restriction enzymes to cut out the appropriate segment. The nucleotide sequence for transcription may also be generated by annealing and ligating synthetic oligonucleotides or by using synthetic oligonucleotides in a polymerase chain reaction (PCR) to give suitable restriction sites at each end. The nucleotide sequence then is cloned into a vector containing suitable regulatory elements, such as upstream promoter and downstream terminator sequences. Typically, plant transformation vectors include one or more cloned plant coding sequence (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a selectable marker. Such plant transformation vectors typically also contain a promoter, a transcription initiation start site, an RNA processing signal (such as splicing signal sequences), a transcription termination site, and/or a polyadenylation signal. Enhancers and targeting sequences may also be present.

The invention provides nucleic acid molecules likely to cause altered fiber length and plant height in a transformed plant. An important aspect of the present invention is the use of nucleic acid constructs wherein a wall-associated kinase-encoding nucleotide sequence is operably linked to one or more promoters, which drive expression of the wall-associated kinase-encoding sequence in a constitutive manner or in certain cell types, organs, or tissues so as to alter the fiber length of a transformed plant compared to the fiber length of a non-transgenic plant.

Suitable constitutive plant promoters which can be useful for expressing the wall-associated kinase sequences suitable for the present invention include but are not limited to the cauliflower mosaic virus (CaMV) 35S promoter, the maize and the *Populus* polyubiquitin promoters, which confer constitutive, high-level expression in most plant tissues (see, e.g., WO 2007/00611, U.S. Pat. No. 5,510,474; Odell et al., *Nature*, 1985, 313: 810-812); the nopaline synthase promoter (An et al., 1988, *Plant Physiol.* 88: 547-552); the FMV promoter from figwort mosaic virus (U.S. Pat. No. 5,378,619) and the octopine synthase promoter (Fromm et al., 1989, *Plant Cell* 1: 977-984).

The promoter can also be chosen so that the expression occurs at a determined time point in the plant’s development, or at a time point determined by outside influences, or in a tissue-specific or tissue-preferred manner. For example, it may ensure specific or preferred expression in fibers cells (cotton fiber-, xylem fiber-, or extra xylary fiber-specific or -preferred promoters).

Exemplary cotton fiber-specific or -preferred promoters include, for example, the cotton CFACT1 gene promoter (U.S. Pat. No. 6,995,256); the E6 gene promoter (U.S. Pat. No. 6,096,950, John et al., 1996, *Plant Mol. Biol.* 30: 297-306; John et al., 1996, *Proc. Natl. Acad. Sci.* 93: 12768-12773); H6 gene promoter (John et al., 1995, *Plant Physiol.* 108: 669-676); GhTUB1 gene promoter (Li et al., 2002, *Plant Physiol.* 130: 666-674) and FbL2A (Rinehart et al., 1996, *Plant Physiol.* 112: 1331-1341 and John et al., 1996, *Proc. Natl. Acad. Sci. USA* 93: 12768-12773).

Vascular system-preferred or -specific promoters, such as xylem-preferred promoters, may be useful for effecting expression of nucleic acid molecules within the invention, specifically in vascular tissue, especially xylem tissue. Thus,

“xylem-preferred” means that the nucleic acid molecules of the current invention are more active in the xylem than in any other plant tissue. The selected promoter should cause the overexpression of the wall-associated kinase, pursuant to the invention, thereby to modify the length of the cell xylem, to modify the height of the host plant, or both.

Suitable promoters are illustrated by but are not limited to the xylem-preferred tubulin (TUB) gene promoter, the caffeic acid 3-O-methyltransferase gene promoter (COMT), the sucrose synthase gene promoter (SuSy), and the xylem-preferred coumarate-4-hydroxylase (C4H) gene promoter. Other suitable xylem-preferred promoters are disclosed in international patent application WO 2005/096805, which is incorporated here by reference.

Synthetic promoters including specific nucleotide regions conferring tissue-specific or tissue-preferred expression may also be used, as exemplified by identification of regulatory elements within larger promoters conferring xylem-preferred expression. Seguin et al., 1997, *Plant Mol. Biol.* 35: 281-291; Torres-Schumann et al., 1996, *Plant J.* 9: 283-296; and Leyva et al., 1992, *Plant Cell* 4: 263-271.

Although the gene expression rate is mainly modulated by the promoter, improvement in expression may also be achieved by the identification and use of enhancer sequences, such as intronic portions of genes, which elevate the expression level of the nearby located genes in an independent manner orientation. In plants, the inclusion of some introns in gene constructs in a position between the promoter and the gene coding sequence leads to increases in mRNA and protein accumulation. Introns known to elevate expression in plants have been identified in maize genes, for example, hsp70, tubA1, Adh1, Sh1, UbH (Brown and Santino, U.S. Pat. Nos. 5,424,412 and 5,859,347; Jeon et al., 2000, *Plant Physiol.* 123: 1005-1014; Callis et al., 1987, *Genes Dev.* 1: 1183-1200; Vasil et al., 1989, *Plant Physiol.* 91: 1575-1579), and in dicotyledonous plant genes such as rbcS from petunia (Dean et al., 1989, *Plant Cell* 1: 201-208); ST-LS1 from potato (Leon et al., 1991, *Plant Physiol.* 95: 968-972) and UBQ3 (Norris et al., 1993, *Plant Mol. Biol.* 21: 895-906) and PAT1 from *Arabidopsis thaliana* (Rose and Last, 1997, *Plant J.* 11: 455-464).

In accordance with one aspect of the invention, a wall-associated kinase sequence is incorporated into a nucleic acid construct that is suitable for plant transformation. Accordingly, nucleic acid constructs are provided comprising a wall-associated kinase sequence, under the control of a transcriptional initiation region operative in a plant, so that the construct can generate RNA in a host plant cell. Preferably, the transcriptional initiation region is part of a vascular or xylem-preferred promoter, such as any of those mentioned above. Such a nucleic acid construct can be used to modify wall-associated kinase gene expression in plants, as described above.

Expression vectors may also contain a selection marker by which transformed cells can be identified in culture. The marker may be associated with the heterologous nucleic acid molecule, i.e., the gene operably linked to a promoter. As used herein, the term “marker” refers to a gene encoding a trait or a phenotype that permits the selection of, or the screening for, a plant or cell containing the marker. In plants, for example, the marker gene will encode antibiotic or herbicide resistance. This allows for selection of transformed cells from among cells that are not transformed or transfected.

Examples of suitable selectable markers include adenosine deaminase, dihydrofolate reductase, hygromycin-B-phosphotransferase, thymidine kinase, xanthine-guanine phospho-ribosyltransferase, glyphosate and glufosinate resis-

tance, and amino-glycoside 3'-O-phosphotransferase (kanamycin, neomycin and G418 resistance). These markers may include resistance to G418, hygromycin, bleomycin, kanamycin, and gentamicin. The construct also may contain the selectable marker gene Bar, which confers resistance to herbicidal phosphinothricin analogs like ammonium glufosinate. Thompson et al., *EMBO J.* 6: 2519-23 (1987). Other suitable selection markers are known as well.

Visible markers such as green fluorescent protein (GFP) may be used. Methods for identifying or selecting transformed plants based on the control of cell division have also been described. See John and Van Mellaert, WO 2000/052168, and Fabijansk et al., WO 2001/059086.

Replication sequences, of bacterial or viral origin, may also be included to allow the vector to be cloned in a bacterial or phage host. Preferably, a broad host range prokaryotic origin of replication is used. A selectable marker for bacteria may be included to allow selection of bacterial cells bearing the desired construct. Suitable prokaryotic selectable markers also include resistance to antibiotics such as kanamycin or tetracycline.

Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art. For instance, when *Agrobacterium* is the host, T-DNA sequences may be included to facilitate the subsequent transfer to and incorporation into plant chromosomes.

Plants for Genetic Engineering

The present invention comprehends the genetic manipulation of plants, especially hardwood trees, to overexpress a wall-associated kinase in vascular tissues via introducing a wall-associated gene, preferably under the control of a xylem-preferred or xylem-specific promoter. The result is enhanced fiber length and plant height.

In this description, the term “plant” denotes any fiber-containing plant material that can be genetically manipulated, including but not limited to differentiated or undifferentiated plant cells, protoplasts, whole plants, plant tissues, or plant organs, or any component of a plant such as a leaf, stem, root, bud, tuber, fruit, rhizome, or the like.

Plants that can be engineered in accordance with the invention include but are not limited to trees, such as *Eucalyptus* species (*E. alba*, *E. albens*, *E. amygdalina*, *E. aromaphloia*, *E. baileyana*, *E. balladoniensis*, *E. bicostata*, *E. botryoides*, *E. brachyandra*, *E. brassiana*, *E. brevistylis*, *E. brockwayi*, *E. camaldulensis*, *E. ceracea*, *E. cloeziana*, *E. coccifera*, *E. cordata*, *E. cornuta*, *E. corticosa*, *E. crebra*, *E. croajingolensis*, *E. curtisii*, *E. dalrympleana*, *E. deglupta*, *E. delegatensis*, *E. delicata*, *E. diversicolor*, *E. diversifolia*, *E. dives*, *E. dolichocarpa*, *E. dundasii*, *E. dunnii*, *E. elata*, *E. erythrocorys*, *E. erythrophloia*, *E. eudesmoides*, *E. falcata*, *E. gamophylla*, *E. glaucina*, *E. globulus*, *E. globulus* subsp. *bicostata*, *E. globulus* subsp. *globulus*, *E. gongylocarpa*, *E. grandis*, *E. grandis* × *urophylla*, *E. guilfoylei*, *E. gunnii*, *E. hallii*, *E. houseana*, *E. jacksonii*, *E. lansdowneana*, *E. latisinensis*, *E. leucophloia*, *E. leucoxydon*, *E. lockyeri*, *E. lucasii*, *E. maidenii*, *E. marginata*, *E. megacarpa*, *E. melliodora*, *E. michaeliana*, *E. microcorys*, *E. microtheca*, *E. muelleriana*, *E. nitens*, *E. nitida*, *E. obliqua*, *E. obtusiflora*, *E. occidentalis*, *E. optima*, *E. ovata*, *E. pachyphylla*, *E. pauciflora*, *E. pellita*, *E. perriniana*, *E. petiolaris*, *E. pilularis*, *E. piperita*, *E. platyphylla*, *E. polyanthemus*, *E. populnea*, *E. preissiana*, *E. pseudo globulus*, *E. pulchella*, *E. radiata*, *E. radiata* subsp. *radiata*, *E. regnans*, *E. risdonii*, *E. robertsonii*, *E. rodwayi*, *E. rubida*, *E. rubiginosa*, *E. saligna*, *E. salmonophloia*, *E. scoparia*, *E. sieberi*, *E. spathulata*, *E. staeri*, *E. stoatei*, *E. tenuipes*, *E. tenuiramis*, *E. tereticornis*, *E. tetragona*, *E. tetradonta*, *E. tindaliae*, *E. torquata*, *E. umbra*, *E. urophylla*, *E. vernicosa*, *E. viminialis*,

E. wandoo, *E. wetarensis*, *E. willisii*, *E. willisii* subsp. *falciformis*, *E. willisii* subsp. *willisii*, *E. woodwardii*), *Populus* species (*P. alba*, *P. alba*×*P. grandidentata*, *P. alba*×*P. tremula*, *P. alba*×*P. tremula* var. *glandulosa*, *P. alba*×*P. tremuloides*, *P. balsamifera*, *P. balsamifera* subsp. *trichocarpa*, *P. balsamifera* subsp. *trichocarpa*×*P. deltoides*, *P. ciliata*, *P. deltoides*, *P. euphratica*, *P. euramericana*, *P. kitakamiensis*, *P. lasiocarpa*, *P. laurifolia*, *P. maximowiczii*, *P. maximowiczii*×*P. balsamifera* subsp. *trichocarpa*, *P. nigra*, *P. sieboldii*×*P. grandidentata*, *P. suaveolens*, *P. szechuanica*, *P. tomentosa*, *P. tremula*, *P. tremula*×*P. tremuloides*, *P. tremuloides*, *P. wilsonii*, *P. canadensis*, *P. yunnanensis*), Conifers such as loblolly pine (*Pinus taeda*), slash pine (*Pinus elliotii*), ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), and Monterey pine (*Pinus radiata*); Douglas-fir (*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and Alaska yellow-cedar (*Chamaecyparis nootkatensis*).

Fiber-producing plants also are included in this context. Illustrative crops are cotton (*Gossipium* spp.), flax (*Linum usitatissimum*), stinging nettle (*Urtica dioica*), hop (*Humulus lupulus*), lime trees (*Tilia cordata*, *T. europaea* and *T. platyphyllus*), spanish broom (*Spartium junceum*), ramie (*Boehmeria nivea*), paper mulberry (*Broussonetia papyrifera*), New Zealand flax (*Phormium tenax*), dogbane (*Apocynum cannabinum*), *Iris* species (*I. douglasiana*, *I. macrosiphon* and *I. purdyi*), milkweeds (*Asclepias* species), pineapple, banana and others. Also contemplated are forage crops, such as alfalfa, lolium, festuca and clover.

In the present description, “transgenic plant” refers to a plant that has incorporated a nucleic acid sequence, including but not limited to genes that are not normally present in a host plant genome, nucleic acid sequences not normally transcribed into RNA or translated into a protein, or any other genes or nucleic acid sequences that one desires to introduce into the wild-type plant, such as genes that normally may be present in the wild-type plant but that one desires either to genetically engineer or to have altered expression. The “transgenic plant” category includes both a primary transformant and a plant that includes a transformant in its lineage, e.g., by way of standard introgression or another breeding procedure.

A “hybrid plant” refers to a plant or a part thereof resulting from a cross between two parent plants, wherein one parent is a genetically engineered plant of the invention. Such cross can occur naturally by, for example, sexual reproduction, or artificially by, for example, in vitro nuclear fusion. Methods of plant breeding are well-known and within the level of one of ordinary skill in the art of plant biology.

In contrast, a plant that is not genetically manipulated is a control plant and is referred to as a “non-transgenic” or “control” plant. Non-transgenic plant can be a plant which genome is neither modified by the introduction of a construct comprising the polynucleotide sequences or fragment thereof of the present invention. It can also be a plant regenerated from cultured cells or tissues without prior modification by the introduction of a construct comprising the polynucleotide sequence of the invention, or may comprise a homozygote recessive progeny (i.e., do not have any copy of the transgene) resulting from self-fertilization of a transgenic plant.

It is contemplated that, in some instances, the genome of an inventive transgenic plant will have been augmented through the stable introduction of a transgene. In other instances, however, the introduced gene will replace an endogenous sequence. A preferred gene in the regard, pursuant to the

present invention, is a wall-associated kinase DNA sequence, for example, one obtained from *Arabidopsis thaliana*.

Methods for Genetic Engineering

Constructs according to the invention may be introduced into any plant cell, using a suitable technique. Both monocotyledonous and dicotyledonous angiosperm or gymnosperm plant cells may be genetically engineered in various ways known to the art. For example, see Klein et al., 1993, *Biotechnology* 4: 583-590; Bechtold et al., 1993, *C. R. Acad. Sci. Paris* 316: 1194-1199; Koncz and Schell, 1986, *Mol. Gen. Genet.* 204: 383-396; Paszkowski et al., 1984, *EMBO J.* 3: 2717-2722; Sagi et al., 1994, *Plant Cell Rep.* 13: 262-266.

Agrobacterium species such as *A. tumefaciens* and *A. rhizogenes* can be used, for example, in accordance with Nagel et al., 1990, *Microbiol Lett* 67: 325. In brief, *Agrobacterium* may be used with a plant expression vector via, e.g., electroporation, after which the *Agrobacterium* is introduced to plant cells via, e.g., the well known leaf-disk method.

Additional methods for accomplishing this include, but are not limited to, transformation by *Rhizobium*, *Sinorhizobium* or *Mesorhizobium* (Broothaerts et al., 2005, *Nature* 433: 629-633), electroporation, particle gun bombardment, calcium phosphate precipitation, and polyethylene glycol fusion, transfer into germinating pollen grains, direct transformation (Lorz et al., 1985, *Mol. Genet.* 199: 179-182), and other methods known to the art. If a selection marker, such as kanamycin resistance, is employed, it makes it easier to determine which cells have been successfully transformed.

The *Agrobacterium* transformation methods discussed above are known to be useful for transforming dicots. Additionally, de la Pena et al., 1987, *Nature* 325: 274-276; Rhodes et al., 1988, *Science* 240: 204-207; and Shimamoto et al., 1989, *Nature* 328: 274-276, all of which are incorporated by reference, have transformed cereal monocots using *Agrobacterium*. Also see Bechtold and Pelletier, 1998, *Methods Mol. Biol.* 82: 259-266, showing the use of vacuum infiltration for *Agrobacterium*-mediated transformation.

The presence of a protein, polypeptide, or nucleic acid molecule in a particular cell can be measured to determine if, for example, a cell has been successfully transformed or transfected. The ability to carry out such assay is well known and need not be reiterated here.

Quantifying Fiber Length and Plant Height

The word “fiber” is often used to unify a diverse group of plant cell types that share in common the features of having an elongated shape and abundant cellulose in thick cell walls, usually, but not always, described as secondary walls. Such walls may or may not be lignified, and the protoplast of such cells may or may not remain alive at maturity. In some industries, the term “fiber” is usually inclusive of thick-walled conducting cells such as vessels and tracheids and to fibrillar aggregates of many individual fiber cells. For the purposes of the present invention, the term “fiber” includes: (a) conducting and non-conducting cells of the xylem; (b) fibers of extraxillary origin, including those from phloem, bark, ground tissue, and epidermis; and (c) fibers from stems, leaves, roots, seeds, and flowers or inflorescences.

Transgenic plants of the invention are characterized by increased fiber length and preferably increased height as well. Increased fiber length in the genetically engineered plant is preferably achieved via WAK overexpression in the plant tissues wherein cell expansion occurs. In describing a plant of the invention, “increased fiber length” refers to a quantitative augmentation in the length of fiber cells in the plant when compared to the length of fiber cells in a wild-type plant”. A quantitative increase of fiber length can be measured by several techniques, such as digitizing, the Kajaani procedure, and

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the Fiber Quality Analyzer. Han et al., 1999, In: Kenaf Properties, Processing and Products, Mississippi State University, Ag & Bio Engineering, pp 149-167.

The fiber length in the engineered plant of the invention is at least from 5 to 15% longer, preferably at least 10-30% and most preferably at least from 20-50% longer than the fiber length of the wild-type plant.

Because increased fiber length can be followed by an increase in plant height, transgenic plants of the invention may have increase fiber length and height. In this description, therefore, the phrase "increased plant height" connote a quantitative increase in plant height, when compared to the height of a wild-type plant. The height in the engineered plant of the invention can be increased to levels of about 5% to about 90%, preferably about 10% to about 75%, even more preferably about 15% to about 65% of the height of the wild-type plant.

* * *

Specific examples are presented below of methods for obtaining wall-associated kinase genes, as well as for introducing the target gene, via *Agrobacterium*, to produce plant transformants. They are meant to be exemplary and not as limitations on the present invention.

EXAMPLE 1

Isolation of a Wall-Associated Kinase DNA Sequence from *Arabidopsis thaliana*(a) RNA Preparation from *Arabidopsis thaliana* Stem and cDNA Synthesis

Stem cuttings of three-months-old *Arabidopsis thaliana* plants were cut in small pieces, frozen in liquid nitrogen, and used for RNA extraction via the cetyltrimethyl-ammonium bromide (CTAB) extraction method. Aldrich and Cullis, 1993, *Plant Mol. Biol. Report*, 11: 128-141. A cDNA pool was used in RT-PCR experiments in which the isolated total RNA was used as template, and Superscript II reverse transcriptase (Invitrogen) and oligo(dT) primer were used to synthesize the first-strand cDNA. Double-stranded cDNA was obtained by the subsequent polymerase reaction, using gene-specific primers, as described below.

(b) Primer Design

A cDNA sequence representing the wall-associated kinase 4 mRNA from *Arabidopsis thaliana* has been determined and deposited in the GenBank under accession number NM101974. Based on this sequence, DNA oligomers were synthesized as primers for PCR, including either the region around the first codon ATG or around the termination codon of the main ORF encoding the wall-associated kinase 4.

Primers were designed to amplify the entire coding region of the wall-associated kinase 4 ORF, i.e., from the ATG through the translation stop codon. The sequences of the primers are given below:

WAK_NDE Length: 23 SEQ ID NO: 11
CATATGAAAGTGCAGCGTCTGTT

WAK_XBA Length: 23 SEQ ID NO: 12
CTAGATCAGCGGCTGCTCAA

(c) PCR Amplification

The cDNA sample obtained in (a) was used as template, and the primers designed in (b) were used for PCR. The PCR steps involved 40 cycles of 1 minute at 94° C., 1 minute at 50°

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C., and 2 minutes at 72° C. followed by an extra step of elongation at 72° C. for 7 minutes. The PCR products were isolated by gel electrophoresis on 1.0% agarose followed by ethidium bromide staining of the electrophoresed gel and detection of amplified bands on a UV transilluminator. The detected amplified band was verified and cut out of the agarose gel with a razor. The pieces of gel were transferred to 1.5 mL microtubes, and the DNA fragments were isolated and purified using a GFX PCR clean-up and gel band purification kit (Amersham). The recovered DNA fragments were sub-cloned to the pGEM-T cloning vector (Promega), transformed into *E. coli*, and then used to prepare plasmid DNA in the usual manner, which was then sequenced by the dideoxy method (Messing, 1983, *Methods in Enzymol.* 101: 20-78), using BigDye chemistry (Applied Biosystems), to yield the DNA sequence disclosed here as SEQ ID NO: 1, for use pursuant to the present invention.

EXAMPLE 2

Preparation of Transgenic *Nicotiana tabacum* Plants

The wall-associated kinase gene obtained in Example 1 above was introduced into a plant host to produce transgenic *Nicotiana tabacum* plants.

(a) Preparation of Constructs and Transformation of *Agrobacterium*

Expression constructs were prepared by cleaving the wall-associated kinase gene obtained in Example 1 above with suitable restriction enzymes so as to include all of the open reading frame and inserting the gene into the plant transformation vector pALELLYX-WAK (FIG. 1) together with an appropriate promoter. For example, the wall-associated kinase gene obtained in Example 1 was cloned into the aforementioned expression vector downstream to a xylem-preferred tubulin gene (TUB) promoter from *Populus deltoides*, as set forth in international application WO 2005/096805. The resulting expression construct was amplified in *E. coli*, and then transformed by freeze thawing into *Agrobacterium tumefaciens* LBA4404 strain.

(b) *Agrobacterium*-Mediated Transformation of *Nicotiana tabacum*

Transformation of *Nicotiana* sp. was accomplished using the leaf disk method of Horsch et al., 1985, *Science* 227: 1229, using a nucleic acid construct comprising the wall-associated kinase gene obtained in (a) operably linked to the TUB promoter of a xylem-preferred gene. The transformants were selected on Murashige and Skoog medium (Sigma, St. Louis, Mo.) containing 100 milligrams/liter of kanamycin and 500 mg/L carbenicillin (Sigma). The transformed tobacco shoots were allowed to root on the Murashige and Skoog medium, and were subsequently transferred to soil and grown in the greenhouse.

(c) PCR Verification of Foreign Gene Insertion into the Host Plant Genome

PCR can be used to verify the integration of the gene construct in the genome of transgenic plants. The PCR reaction mixture contained 100 ng genomic DNA of transformed plant, and 0.2 μM of each primer described above, 100 μM of each deoxyribonucleotide triphosphate, 5 μL PCR buffer and 2.5 Units of AmpliTaq DNA polymerase (Applied Biosystems) in a total volume of 50 μL. The cycling parameters were as follows: 94° C. for 1 minute, 50° C. for 1 minute and 72° C. for 3 minutes, for 40 cycles, with 5 minutes at 72° C. extension. The PCR products were electrophoresized on a 1% agarose gel.

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(d) Determination of Transgene Expression Level in Transgenic Plants

Semi-quantitative RT-PCR was used to detect the accumulation of wall-associated kinase transcripts in stem tissue of the transgenic plants. Total RNA was isolated from stem cuts of 3-months old transgenic *Nicotiana* T0 and T1 plants using the CTAB method described by Aldrich and Cullis, 1993, *Plant Mol. Biol. Report.* 11: 128-141.

cDNA was synthesized from 500 ng of total RNA using Superscript II RNase H-RT (Invitrogen, USA). The primers described above were used along with primers for the constitutive gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control to normalize the quantity of total RNA used in each sample. PCR was done with a 12.5-fold dilution of the first-strand cDNA under the following conditions: 94° C. for 3 minutes and 27 cycles of 94° C. for 1 minute, 52 to 60° C. for 45 seconds, and 72° C. for 1 minute and 30 seconds.

EXAMPLE 3

Increase in Fiber Length in Tobacco Transgenic Plants Overexpressing Wall-Associated Kinase Gene in Vascular Tissues

Stem regions corresponding to 50% height of transgenic and control plants of 5 months old were macerated in acetic acid-peroxide solution at 70° C. for 48 hours or until single cells were obtained. Cells were stained with safranin and examined under a microscope (Leica DMIL) fitted with a camera (Sony) linked to a personal computer. Cells (about 100 per line) were measured directly on the screen, using the "Image Tool" software.

Three of the transgenic events, known to express the transgene according to procedure detailed in Example 2, showed a statistically significant increase in fiber length (FIG. 2). Transgenic event 43B exhibits an increase of 21% in fiber length as compared to the control plants ($P < 0.05$, t-test). Transgenic event 47B exhibits an increase of 19% in fiber length when compared to the control plants (FIG. 2; $P < 0.05$, t-test). Additionally, transgenic event 43B exhibit an increase of 15% in fiber length as compared to the control plants (FIG. 2 $P < 0.05$, t-test).

It is important to mention that another strategy to increase fiber length by the overexpression of a pectin methyl esterase gene (Berthold et al., WO 2006/068603) has achieved an increase of only 5% on fiber length of transgenic plants when compared to control plants.

After grown to maturity, the T0 events were selfed to generate T1 lines. Plants that are homozygote dominant present a significant increase of 10% in fiber length ($P < 0.05$, t-test), when compared to homozygote recessive plants. These results were observed in two different lines (FIG. 3 and FIG. 4).

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EXAMPLE 4

Increase in Plant Height in Tobacco Transgenic Plants Overexpressing Wall-Associated Kinase Gene in Vascular Tissues

T₁ progeny resulting from self-fertilization of transgenic plants was individually potted 3 weeks after sowing. Growth was measured periodically until the first flower was formed (plants were about 5 months old), and was recorded as total height.

The results presented are an example of the increase in plant height observed in the homozygote dominant plants of different lines. Plant height of the three genotypes from the event 51B was compared. Plants that are homozygote dominant are 12% higher than the homozygote recessive plants. Plants that are hemizygote are 9% higher than the homozygote recessive plants ($P < 0.05$, t-test) (FIG. 5).

EXAMPLE 5

Preparation of Transgenic *Populus* Plants

The gene obtained in Example 1 above was introduced into a plant host to produce transgenic *Populus* plants.

(a) Preparation of Constructs and Transformation of *Agrobacterium*

Expression constructs can be prepared by cleaving the wall-associated kinase gene obtained in Example 1 above with suitable restriction enzymes so as to include the entire open reading frame and inserting the gene into the plant transformation vector pALELLYX-WAK (FIG. 1) together with an appropriate promoter. For example, the wall-associated kinase gene obtained in Example 1 was cloned into the aforementioned expression vector downstream to a xylem-preferred tubulin gene (TUB) promoter from *Populus deltoides*, as set forth in international application WO 2005/096805. The resulting expression construct was amplified in *E. coli*, and then transformed by freeze thawing into *Agrobacterium tumefaciens* LBA4404 strain.

(b) *Agrobacterium*-Mediated Transformation of *Populus*

Wild-type aspen was transformed with *Agrobacterium tumefaciens* carrying a construct comprising an *Arabidopsis thaliana* wall-associated kinase gene obtained in Example 1 operably linked to the promoter of a xylem-preferred gene (TUB). Petioles and internodal stem segments from in vitro micropropagated plants were used as explants. Transformed shoots are selected on regeneration medium containing 100 mg/L of kanamycin and allowed to root on the Murashige and Skoog medium. Selected plants are subsequently transferred to soil and grown in the greenhouse.

SEQUENCE LISTING

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<211> LENGTH: 2217

<212> TYPE: DNA

<213> ORGANISM: *Arabidopsis thaliana*

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(2214)

<223> OTHER INFORMATION: Wall-associated kinase 4, cDNA, complete CD

-continued

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1 5 10 15	
atg cag ctg gtc aag ggg caa acc ttg cct cgt tgc ccc gaa aaa tgt	96
Met Gln Leu Val Lys Gly Gln Thr Leu Pro Arg Cys Pro Glu Lys Cys	
20 25 30	
ggc aac gtt aca ctt gag tac cct ttt ggc ttt tct cca ggt tgt tgg	144
Gly Asn Val Thr Leu Glu Tyr Pro Phe Gly Phe Ser Pro Gly Cys Trp	
35 40 45	
cgt gcc gaa gat cct agt ttc aat ctc agt tgt gtg aac gag aat cta	192
Arg Ala Glu Asp Pro Ser Phe Asn Leu Ser Cys Val Asn Glu Asn Leu	
50 55 60	
ttc tat aag ggc ctt gaa gtg gtc gaa ata tct cac agc agc cag tta	240
Phe Tyr Lys Gly Leu Glu Val Val Glu Ile Ser His Ser Ser Gln Leu	
65 70 75 80	
cgc gtc cta tat cct gca tcc tac att tgc tac aac agc aaa gga aag	288
Arg Val Leu Tyr Pro Ala Ser Tyr Ile Cys Tyr Asn Ser Lys Gly Lys	
85 90 95	
ttc gct aaa ggg act tac tac tgg agt aat cta ggt aat ttg act ctc	336
Phe Ala Lys Gly Thr Tyr Tyr Trp Ser Asn Leu Gly Asn Leu Thr Leu	
100 105 110	
tcc ggc aac aac acg att act gca tta ggc tgt aac tcg tac gct ttt	384
Ser Gly Asn Asn Thr Ile Thr Ala Leu Gly Cys Asn Ser Tyr Ala Phe	
115 120 125	
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Val Ser Ser Asn Gly Thr Arg Arg Asn Ser Val Gly Cys Ile Ser Ala	
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Cys Asp Ala Leu Ser His Glu Ala Asn Gly Glu Cys Asn Gly Glu Gly	
145 150 155 160	
tgc tgc cag aac ccc gtc cct gca ggg aac aat tgg tta ata gtc aga	528
Cys Cys Gln Asn Pro Val Pro Ala Gly Asn Asn Trp Leu Ile Val Arg	
165 170 175	
tca tat cgc ttt gac aac gac acg tca gtg caa cct atc tct gag ggt	576
Ser Tyr Arg Phe Asp Asn Asp Thr Ser Val Gln Pro Ile Ser Glu Gly	
180 185 190	
caa tgc atc tac gcc ttt ctc gtt gaa aat ggc aag ttt aag tac aat	624
Gln Cys Ile Tyr Ala Phe Leu Val Glu Asn Gly Lys Phe Lys Tyr Asn	
195 200 205	
gct tcg gac aaa tat tct tat ctg cag aat agg aat gtg ggg ttt cct	672
Ala Ser Asp Lys Tyr Ser Tyr Leu Gln Asn Arg Asn Val Gly Phe Pro	
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Val Val Leu Asp Trp Ser Ile Arg Gly Glu Thr Cys Gly Gln Val Gly	
225 230 235 240	
gaa aag aaa tgc ggt gtg aat ggc ata tgt tcc aat tct gct agt ggg	768
Glu Lys Lys Cys Gly Val Asn Gly Ile Cys Ser Asn Ser Ala Ser Gly	
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atc ggg tat aca tgc aaa tgc aaa gga ggt ttc cag ggg aat cca tat	816
Ile Gly Tyr Thr Cys Lys Cys Lys Gly Gly Phe Gln Gly Asn Pro Tyr	
260 265 270	
ctt caa aac ggt tgc caa gac atc aat gag tgt act act gct aat cct	864
Leu Gln Asn Gly Cys Gln Asp Ile Asn Glu Cys Thr Thr Ala Asn Pro	
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Ile His Lys His Asn Cys Ser Gly Asp Ser Thr Cys Glu Asn Lys Leu	
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Gly His Phe Arg Cys Asn Cys Arg Ser Arg Tyr Glu Leu Asn Thr Thr	
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Thr Asn Thr Cys Lys Pro Lys Gly Asn Pro Glu Tyr Val Glu Trp Thr	
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Ile Ser Cys Ile Glu His Lys Met Lys Asn Thr Lys Asp Thr Glu Leu	
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Arg Gln Gln Phe Phe Glu Gln Asn Gly Gly Gly Met Leu Met Gln Arg	
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Leu Ser Gly Ala Gly Pro Ser Asn Val Asp Val Lys Ile Phe Thr Glu	
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Gly Gln Gly Gly Gln Gly Thr Val Tyr Lys Gly Ile Leu Pro Asp Asn	
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Glu Gln Phe Ile Asn Glu Val Leu Val Leu Ser Gln Ile Asn His Arg	
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Tyr Leu Asp Pro Glu Tyr Tyr Asn Thr Gly Leu Leu Asn Glu Lys Ser	
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Lys Ala Leu Cys Phe Glu Arg Pro Gln Thr Ser Lys His Ile Val Ser	
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Gly	Asn	Val 35	Thr	Leu	Glu	Tyr	Pro 40	Phe	Gly	Phe	Ser	Pro 45	Gly	Cys	Trp
Arg	Ala 50	Glu	Asp	Pro	Ser	Phe 55	Asn	Leu	Ser	Cys	Val 60	Asn	Glu	Asn	Leu
Phe 65	Tyr	Lys	Gly	Leu	Glu 70	Val	Val	Glu	Ile	Ser 75	His	Ser	Ser	Gln	Leu 80
Arg	Val	Leu	Tyr 85	Pro	Ala	Ser	Tyr	Ile	Cys 90	Tyr	Asn	Ser	Lys	Gly 95	Lys
Phe	Ala	Lys 100	Gly	Thr	Tyr	Tyr	Trp	Ser 105	Asn	Leu	Gly	Asn 110	Leu	Thr	Leu
Ser	Gly 115	Asn	Asn	Thr	Ile	Thr	Ala 120	Leu	Gly	Cys	Asn 125	Ser	Tyr	Ala	Phe
Val 130	Ser	Ser	Asn	Gly	Thr	Arg 135	Arg	Asn	Ser	Val	Gly 140	Cys	Ile	Ser	Ala
Cys 145	Asp	Ala	Leu	Ser	His 150	Glu	Ala	Asn	Gly	Glu 155	Cys	Asn	Gly	Glu	Gly 160
Cys	Cys	Gln	Asn 165	Pro	Val	Pro	Ala	Gly	Asn 170	Asn	Trp	Leu	Ile	Val 175	Arg
Ser	Tyr	Arg 180	Phe	Asp	Asn	Asp	Thr	Ser 185	Val	Gln	Pro	Ile 190	Ser	Glu	Gly
Gln	Cys	Ile 195	Tyr	Ala	Phe	Leu	Val 200	Glu	Asn	Gly	Lys 205	Phe	Lys	Tyr	Asn

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Glu	Lys	Lys	Cys	Gly	Val	Asn	Gly	Ile	Cys	Ser	Asn	Ser	Ala	Ser	Gly
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Thr	Asn	Thr	Cys	Lys	Pro	Lys	Gly	Asn	Pro	Glu	Tyr	Val	Glu	Trp	Thr
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Leu	Val	Tyr	Glu	Phe	Ile	Ser	Ser	Gly	Thr	Leu	Phe	Asp	His	Leu	His
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Gly	Ser	Met	Phe	Asp	Ser	Ser	Leu	Thr	Trp	Glu	His	Arg	Leu	Arg	Met
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Ala	Val	Glu	Ile	Ala	Gly	Thr	Leu	Ala	Tyr	Leu	His	Ser	Ser	Ala	Ser
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Pro	Met	Asp	Lys	Glu</											

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Arg Ile Ala Val Glu Cys Thr Arg Leu Thr Gly Glu Glu Arg Pro Gly	660	665	670
Met Lys Glu Val Ala Ala Glu Leu Glu Ala Leu Arg Val Thr Lys Thr	675	680	685
Lys His Lys Trp Ser Asp Glu Tyr Pro Glu Gln Glu Asp Thr Glu His	690	695	700
Leu Val Gly Val Gln Lys Leu Ser Ala Gln Gly Glu Thr Ser Ser Ser	705	710	715
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Gln Asn Lys Cys Gly Asn Ile Thr Ile Glu Tyr Pro Phe Gly Ile Ser			
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Glu Asp Arg Pro His Val Leu Ser Asp Ile Glu Val Ala Asn Phe Asn			
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cac agc ggc cag cta caa gtt ctg ctt aat cga tcc tct act tgc tac			288
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85 90 95			
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Asp Glu Gln Gly Lys Lys Thr Glu Glu Asp Ser Ser Phe Thr Leu Glu			
100 105 110			
aat tta tct ctt tcc gcc aac aac aag tta act gca gta ggc tgt aac			384
Asn Leu Ser Leu Ser Ala Asn Asn Lys Leu Thr Ala Val Gly Cys Asn			
115 120 125			
gct tta tca ctt ctg gac act ttt gga atg caa aac tac tca act gca			432
Ala Leu Ser Leu Leu Asp Thr Phe Gly Met Gln Asn Tyr Ser Thr Ala			
130 135 140			
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Cys Leu Ser Leu Cys Asp Ser Pro Pro Glu Ala Asp Gly Glu Cys Asn			
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Gly Arg Gly Cys Cys Arg Val Asp Val Ser Ala Pro Leu Asp Ser Tyr			
165 170 175			
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Asp	Phe	Ser	Pro	Cys	Thr	Tyr	Ala	Phe	Leu	Val	Glu	Asp	Asp	Lys	Phe	
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aac	ttc	agt	tct	aca	gaa	gat	ctt	ctg	aat	ctg	cga	aat	gtc	atg	agg	672
Asn	Phe	Ser	Ser	Thr	Glu	Asp	Leu	Leu	Asn	Leu	Arg	Asn	Val	Met	Arg	
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Phe	Pro	Val	Leu	Leu	Asp	Trp	Ser	Val	Gly	Asn	Gln	Thr	Cys	Glu	Gln	
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Val	Gly	Ser	Thr	Ser	Ile	Cys	Gly	Gly	Asn	Ser	Thr	Cys	Leu	Asp	Ser	
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Thr	Pro	Arg	Asn	Gly	Tyr	Ile	Cys	Arg	Cys	Asn	Glu	Gly	Phe	Asp	Gly	
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Asn	Pro	Tyr	Leu	Ser	Ala	Gly	Cys	Gln	Asp	Val	Asn	Glu	Cys	Thr	Thr	
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Ser	Ser	Thr	Ile	His	Arg	His	Asn	Cys	Ser	Asp	Pro	Lys	Thr	Cys	Arg	
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Asn	Lys	Val	Gly	Gly	Phe	Tyr	Cys	Lys	Cys	Gln	Ser	Gly	Tyr	Arg	Leu	
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Asp	Thr	Thr	Thr	Met	Ser	Cys	Lys	Arg	Lys	Glu	Phe	Ala	Trp	Thr	Thr	
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gaa	caa	ttc	ttc	gag	caa	aat	ggg	ggc	ggc	atg	ttg	aca	caa	cga	ctc	1152
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Ser Gly Cys Tyr Tyr Pro Gly Asn Glu Ser Phe Ser Ile Thr Cys Lys
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Glu 65	Asp	Arg	Pro	His	Val 70	Leu	Ser	Asp	Ile	Glu 75	Val	Ala	Asn	Phe	Asn 80
His	Ser	Gly	Gln	Leu 85	Gln	Val	Leu	Leu	Asn 90	Arg	Ser	Ser	Thr	Cys 95	Tyr
Asp	Glu	Gln	Gly 100	Lys	Lys	Thr	Glu	Glu 105	Asp	Ser	Ser	Phe	Thr	Leu	Glu
Asn	Leu	Ser	Leu	Ser	Ala	Asn	Asn 120	Lys	Leu	Thr	Ala	Val 125	Gly	Cys	Asn
Ala	Leu 130	Ser	Leu	Leu	Asp	Thr 135	Phe	Gly	Met	Gln	Asn 140	Tyr	Ser	Thr	Ala
Cys 145	Leu	Ser	Leu	Cys	Asp 150	Ser	Pro	Pro	Glu	Ala 155	Asp	Gly	Glu	Cys	Asn 160
Gly	Arg	Gly	Cys	Cys 165	Arg	Val	Asp	Val	Ser 170	Ala	Pro	Leu	Asp	Ser	Tyr
Thr	Phe	Glu	Thr 180	Thr	Ser	Gly	Arg	Ile 185	Lys	His	Met	Thr 190	Ser	Phe	His
Asp	Phe 195	Ser	Pro	Cys	Thr	Tyr	Ala 200	Phe	Leu	Val	Glu	Asp 205	Asp	Lys	Phe
Asn	Phe 210	Ser	Ser	Thr	Glu	Asp 215	Leu	Leu	Asn	Leu	Arg 220	Asn	Val	Met	Arg
Phe 225	Pro	Val	Leu	Leu	Asp 230	Trp	Ser	Val	Gly	Asn 235	Gln	Thr	Cys	Glu	Gln 240
Val	Gly	Ser	Thr 245	Ser	Ile	Cys	Gly	Gly	Asn 250	Ser	Thr	Cys	Leu	Asp 255	Ser
Thr	Pro	Arg	Asn 260	Gly	Tyr	Ile	Cys	Arg 265	Cys	Asn	Glu	Gly 270	Phe	Asp	Gly
Asn	Pro 275	Tyr	Leu	Ser	Ala	Gly	Cys 280	Gln	Asp	Val	Asn	Glu 285	Cys	Thr	Thr
Ser	Ser 290	Thr	Ile	His	Arg	His 295	Asn	Cys	Ser	Asp	Pro 300	Lys	Thr	Cys	Arg
Asn 305	Lys	Val	Gly	Gly	Phe 310	Tyr	Cys	Lys	Cys	Gln 315	Ser	Gly	Tyr	Arg	Leu 320
Asp	Thr	Thr	Thr 325	Met	Ser	Cys	Lys	Arg	Lys 330	Glu	Phe	Ala	Trp	Thr	Thr 335
Ile	Leu	Leu	Val 340	Thr	Thr	Ile	Gly	Phe 345	Leu	Val	Ile	Leu 350	Leu	Gly	Val 355
Ala	Cys 355	Ile	Gln	Gln	Arg	Met	Lys 360	His	Leu	Lys	Asp	Thr 365	Lys	Leu	Arg 370
Glu	Gln 370	Phe	Phe	Glu	Gln	Asn 375	Gly	Gly	Gly	Met	Leu 380	Thr	Gln	Arg	Leu 385
Ser 385	Gly	Ala	Gly	Pro	Ser 390	Asn	Val	Asp	Val	Lys 395	Ile	Phe	Thr	Glu	Asp 400
Gly	Met	Lys	Lys 405	Ala	Thr	Asn	Gly	Tyr	Ala 410	Glu	Ser	Arg	Ile	Leu	Gly 415
Gln	Gly	Gly	Gln 420	Gly	Thr	Val	Tyr	Lys 425	Gly	Ile	Leu	Pro	Asp 430	Asn	Ser 435
Ile	Val 435	Ala	Ile	Lys	Lys	Ala 440	Arg	Leu	Gly	Asp	Ser	Ser 445	Gln	Val	Glu 450
Gln	Phe 450	Ile	Asn	Glu	Val	Leu 455	Val	Leu	Ser	Gln	Ile	Asn 460	His	Arg	Asn 465
Val	Val 465	Lys	Leu	Leu	Gly 470	Cys	Cys	Leu	Glu	Thr	Glu 475	Val	Pro	Leu	Leu 480

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cag ctt cgt gtt cgg cta gtt aga tcc aga gtt tgc tac gat agt caa	288
Gln Leu Arg Val Arg Leu Val Arg Ser Arg Val Cys Tyr Asp Ser Gln	
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gga aaa cag act gac tac att gcc cag cgg acc acc ctg ggt aat ttc	336
Gly Lys Gln Thr Asp Tyr Ile Ala Gln Arg Thr Thr Leu Gly Asn Phe	
100 105 110	
act ctc tct gaa ctt aac aga ttt act gta gta ggt tgt aac agt tac	384
Thr Leu Ser Glu Leu Asn Arg Phe Thr Val Val Gly Cys Asn Ser Tyr	
115 120 125	
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Ala Phe Leu Arg Thr Ser Gly Val Glu Lys Tyr Ser Thr Gly Cys Ile	
130 135 140	
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Ser Ile Cys Asp Ser Ala Thr Thr Lys Asn Gly Ser Cys Ser Gly Glu	
145 150 155 160	
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Gly Cys Cys Gln Ile Pro Val Pro Arg Gly Tyr Ser Phe Val Arg Val	
165 170 175	
aaa cca cat agc ttt cac aac cat cct act gtg cat ctg ttt aat cct	576
Lys Pro His Ser Phe His Asn His Pro Thr Val His Leu Phe Asn Pro	
180 185 190	
tgc acc tac gcc ttt ctc gtt gaa gat ggt atg ttt gac ttc cat gct	624
Cys Thr Tyr Ala Phe Leu Val Glu Asp Gly Met Phe Asp Phe His Ala	
195 200 205	
ttg gaa gat ctc aac aat ctg cga aat gtt act acg ttc cct gta gta	672
Leu Glu Asp Leu Asn Asn Leu Arg Asn Val Thr Thr Phe Pro Val Val	
210 215 220	
cta gat tgg tct atc gga gac aag act tgc aaa caa gta gaa tac agg	720
Leu Asp Trp Ser Ile Gly Asp Lys Thr Cys Lys Gln Val Glu Tyr Arg	
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ggc gtg tgt ggt ggt aac agc aca tgt ttc gat tct act ggt gga acc	768
Gly Val Cys Gly Gly Asn Ser Thr Cys Phe Asp Ser Thr Gly Gly Thr	
245 250 255	
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Gly Tyr Asn Cys Lys Cys Leu Glu Gly Phe Glu Gly Asn Pro Tyr Leu	
260 265 270	
cca aac ggt tgt caa gac atc aat gaa tgt att agt agt aga cat aac	864
Pro Asn Gly Cys Gln Asp Ile Asn Glu Cys Ile Ser Ser Arg His Asn	
275 280 285	
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Cys Ser Glu His Ser Thr Cys Glu Asn Thr Lys Gly Ser Phe Asn Cys	
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Asn Cys Pro Ser Gly Tyr Arg Lys Asp Ser Leu Asn Ser Cys Thr Arg	
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aaa gtc agg cct gaa tac ttt aga tgg act caa att ttt ctt gga acc	1008
Lys Val Arg Pro Glu Tyr Phe Arg Trp Thr Gln Ile Phe Leu Gly Thr	
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acc atc ggc ttc tcg gtt atc atg ctt ggg att agc tgt cta caa cag	1056
Thr Ile Gly Phe Ser Val Ile Met Leu Gly Ile Ser Cys Leu Gln Gln	
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Lys Ile Lys His Arg Lys Asn Thr Glu Leu Arg Gln Lys Phe Phe Glu	
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caa aat ggt gga ggc atg ttg ata cag cga gtc tcg gga gca ggg cca	1152
Gln Asn Gly Gly Gly Met Leu Ile Gln Arg Val Ser Gly Ala Gly Pro	
370 375 380	
tca aat gtt gat gtc aaa atc ttc act gag aaa gga atg aag gaa gca	1200
Ser Asn Val Asp Val Lys Ile Phe Thr Glu Lys Gly Met Lys Glu Ala	
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act aat ggt tac cat gag agc aga atc ctg ggt cag gga ggc caa gga	1248
Thr Asn Gly Tyr His Glu Ser Arg Ile Leu Gly Gln Gly Gly Gln Gly	
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aca gtg tac aaa ggg ata ttg ccg gac aac tcc ata gtt gct ata aag	1296
Thr Val Tyr Lys Gly Ile Leu Pro Asp Asn Ser Ile Val Ala Ile Lys	
420 425 430	
aaa gct cgg ctt gga aac cgt agc caa gta gag cag ttc atc aac gaa	1344
Lys Ala Arg Leu Gly Asn Arg Ser Gln Val Glu Gln Phe Ile Asn Glu	
435 440 445	
gtg cta gtg ctt tca caa atc aac cat agg aac gtg gtc aag gtc ttg	1392
Val Leu Val Leu Ser Gln Ile Asn His Arg Asn Val Val Lys Val Leu	
450 455 460	
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Gly Cys Cys Leu Glu Thr Glu Val Pro Leu Leu Val Tyr Glu Phe Ile	
465 470 475 480	
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Asn Ser Gly Thr Leu Phe Asp His Leu His Gly Ser Leu Tyr Asp Ser	
485 490 495	
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Ser Leu Thr Trp Glu His Arg Leu Arg Ile Ala Thr Glu Val Ala Gly	
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Ser Leu Ala Tyr Leu His Ser Ser Ala Ser Ile Pro Ile Ile His Arg	
515 520 525	
gat atc aag act gct aat att ctc ctg gat aaa aac tta act gca aaa	1632
Asp Ile Lys Thr Ala Asn Ile Leu Leu Asp Lys Asn Leu Thr Ala Lys	
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Val Ala Asp Phe Gly Ala Ser Arg Leu Ile Pro Met Asp Lys Glu Gln	
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565 570 575	
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Tyr Asn Thr Gly Leu Leu Asn Glu Lys Ser Asp Val Tyr Ser Phe Gly	
580 585 590	
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Val Val Leu Met Glu Leu Leu Ser Gly Gln Lys Ala Leu Cys Phe Glu	
595 600 605	
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Arg Pro His Cys Pro Lys Asn Leu Val Ser Cys Phe Ala Ser Ala Thr	
610 615 620	
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Lys Asn Asn Arg Phe His Glu Ile Ile Asp Gly Gln Val Met Asn Glu	
625 630 635 640	
gat aac cag aga gag atc cag gaa gct gca aga att gct gca gag tgt	1968
Asp Asn Gln Arg Glu Ile Gln Glu Ala Ala Arg Ile Ala Ala Glu Cys	
645 650 655	
aca agg cta atg gga gag gaa agg cca agg atg aaa gaa gta gct gca	2016
Thr Arg Leu Met Gly Glu Glu Arg Pro Arg Met Lys Glu Val Ala Ala	
660 665 670	
gag tta gag gcc ttg aga gtt aaa aca act aaa tat aag tgg tcg gat	2064
Glu Leu Glu Ala Leu Arg Val Lys Thr Thr Lys Tyr Lys Trp Ser Asp	
675 680 685	
cag tat cgt gag aca ggg gag att gaa cac ttg ctc ggc gtt caa atc	2112
Gln Tyr Arg Glu Thr Gly Glu Ile Glu His Leu Leu Gly Val Gln Ile	
690 695 700	
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Leu Ser Ala Gln Gly Glu Thr Ser Ser Ser Ile Gly Tyr Asp Ser Ile	

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705	710	715	720	
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<210> SEQ ID NO 6
<211> LENGTH: 732
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 6

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Cys Gly Asn Val Ala Val Glu Tyr Pro Phe Gly Thr Ser Pro Gly Cys
35 40 45
Tyr Tyr Pro Gly Asp Glu Ser Phe Asn Leu Thr Cys Asn Glu Gln Glu
50 55 60
Lys Leu Phe Phe Gly Asn Met Pro Val Ile Asn Met Ser Leu Ser Gly
65 70 75 80
Gln Leu Arg Val Arg Leu Val Arg Ser Arg Val Cys Tyr Asp Ser Gln
85 90 95
Gly Lys Gln Thr Asp Tyr Ile Ala Gln Arg Thr Thr Leu Gly Asn Phe
100 105 110
Thr Leu Ser Glu Leu Asn Arg Phe Thr Val Val Gly Cys Asn Ser Tyr
115 120 125
Ala Phe Leu Arg Thr Ser Gly Val Glu Lys Tyr Ser Thr Gly Cys Ile
130 135 140
Ser Ile Cys Asp Ser Ala Thr Thr Lys Asn Gly Ser Cys Ser Gly Glu
145 150 155 160
Gly Cys Cys Gln Ile Pro Val Pro Arg Gly Tyr Ser Phe Val Arg Val
165 170 175
Lys Pro His Ser Phe His Asn His Pro Thr Val His Leu Phe Asn Pro
180 185 190
Cys Thr Tyr Ala Phe Leu Val Glu Asp Gly Met Phe Asp Phe His Ala
195 200 205
Leu Glu Asp Leu Asn Asn Leu Arg Asn Val Thr Thr Phe Pro Val Val
210 215 220
Leu Asp Trp Ser Ile Gly Asp Lys Thr Cys Lys Gln Val Glu Tyr Arg
225 230 235 240
Gly Val Cys Gly Gly Asn Ser Thr Cys Phe Asp Ser Thr Gly Gly Thr
245 250 255
Gly Tyr Asn Cys Lys Cys Leu Glu Gly Phe Glu Gly Asn Pro Tyr Leu
260 265 270
Pro Asn Gly Cys Gln Asp Ile Asn Glu Cys Ile Ser Ser Arg His Asn
275 280 285
Cys Ser Glu His Ser Thr Cys Glu Asn Thr Lys Gly Ser Phe Asn Cys
290 295 300
Asn Cys Pro Ser Gly Tyr Arg Lys Asp Ser Leu Asn Ser Cys Thr Arg
305 310 315 320
Lys Val Arg Pro Glu Tyr Phe Arg Trp Thr Gln Ile Phe Leu Gly Thr
325 330 335
Thr Ile Gly Phe Ser Val Ile Met Leu Gly Ile Ser Cys Leu Gln Gln
340 345 350

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Lys Ile Lys His Arg Lys Asn Thr Glu Leu Arg Gln Lys Phe Phe Glu
 355 360 365
 Gln Asn Gly Gly Gly Met Leu Ile Gln Arg Val Ser Gly Ala Gly Pro
 370 375 380
 Ser Asn Val Asp Val Lys Ile Phe Thr Glu Lys Gly Met Lys Glu Ala
 385 390 395 400
 Thr Asn Gly Tyr His Glu Ser Arg Ile Leu Gly Gln Gly Gly Gln Gly
 405 410 415
 Thr Val Tyr Lys Gly Ile Leu Pro Asp Asn Ser Ile Val Ala Ile Lys
 420 425 430
 Lys Ala Arg Leu Gly Asn Arg Ser Gln Val Glu Gln Phe Ile Asn Glu
 435 440 445
 Val Leu Val Leu Ser Gln Ile Asn His Arg Asn Val Val Lys Val Leu
 450 455 460
 Gly Cys Cys Leu Glu Thr Glu Val Pro Leu Leu Val Tyr Glu Phe Ile
 465 470 475 480
 Asn Ser Gly Thr Leu Phe Asp His Leu His Gly Ser Leu Tyr Asp Ser
 485 490 495
 Ser Leu Thr Trp Glu His Arg Leu Arg Ile Ala Thr Glu Val Ala Gly
 500 505 510
 Ser Leu Ala Tyr Leu His Ser Ser Ala Ser Ile Pro Ile Ile His Arg
 515 520 525
 Asp Ile Lys Thr Ala Asn Ile Leu Leu Asp Lys Asn Leu Thr Ala Lys
 530 535 540
 Val Ala Asp Phe Gly Ala Ser Arg Leu Ile Pro Met Asp Lys Glu Gln
 545 550 555 560
 Leu Thr Thr Ile Val Gln Gly Thr Leu Gly Tyr Leu Asp Pro Glu Tyr
 565 570 575
 Tyr Asn Thr Gly Leu Leu Asn Glu Lys Ser Asp Val Tyr Ser Phe Gly
 580 585 590
 Val Val Leu Met Glu Leu Leu Ser Gly Gln Lys Ala Leu Cys Phe Glu
 595 600 605
 Arg Pro His Cys Pro Lys Asn Leu Val Ser Cys Phe Ala Ser Ala Thr
 610 615 620
 Lys Asn Asn Arg Phe His Glu Ile Ile Asp Gly Gln Val Met Asn Glu
 625 630 635 640
 Asp Asn Gln Arg Glu Ile Gln Glu Ala Ala Arg Ile Ala Ala Glu Cys
 645 650 655
 Thr Arg Leu Met Gly Glu Glu Arg Pro Arg Met Lys Glu Val Ala Ala
 660 665 670
 Glu Leu Glu Ala Leu Arg Val Lys Thr Thr Lys Tyr Lys Trp Ser Asp
 675 680 685
 Gln Tyr Arg Glu Thr Gly Glu Ile Glu His Leu Leu Gly Val Gln Ile
 690 695 700
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 705 710 715 720
 Arg Asn Val Thr Thr Leu Asp Ile Glu Ala Gly Arg
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<210> SEQ ID NO 7

<211> LENGTH: 2226

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<220> FEATURE:

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<221> NAME/KEY: CDS

<222> LOCATION: (1) .. (2223)

<223> OTHER INFORMATION: Wall-associated kinase 3, cDNA, complete CDS

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1           5           10          15

tat act cag ctt gtg aag ggg caa cat caa cct cgc gaa gat tgt aaa      96
Tyr Thr Gln Leu Val Lys Gly Gln His Gln Pro Arg Glu Asp Cys Lys
          20          25          30

ctt aaa tgt gga aac gtc aca ata gag tac cct ttt ggt att tct aca     144
Leu Lys Cys Gly Asn Val Thr Ile Glu Tyr Pro Phe Gly Ile Ser Thr
          35          40          45

ggt tgt tac tat ccc gga gat gat aat ttc aat ctc acc tgt gtc gtg     192
Gly Cys Tyr Tyr Pro Gly Asp Asp Asn Phe Asn Leu Thr Cys Val Val
          50          55          60

gaa gag aag cta cta ctc ttt ggc atc att caa gtg acc aat att tct     240
Glu Glu Lys Leu Leu Leu Phe Gly Ile Ile Gln Val Thr Asn Ile Ser
        65          70          75          80

cac agt ggc cat gta agt gta ctg ttt gaa cga ttc tct gaa tgc tac     288
His Ser Gly His Val Ser Val Leu Phe Glu Arg Phe Ser Glu Cys Tyr
          85          90          95

gag cag aaa aat gag act aat gga act gcc ctc ggg tat cag ctg ggt     336
Glu Gln Lys Asn Glu Thr Asn Gly Thr Ala Leu Gly Tyr Gln Leu Gly
          100          105          110

agt agt ttc tct ctc tcc tcc aac aac aag ttt act tta gta gga tgt     384
Ser Ser Phe Ser Leu Ser Ser Asn Asn Lys Phe Thr Leu Val Gly Cys
          115          120          125

aac gct tta tca ctt ttg agc act ttt gga aag caa aac tac tca act     432
Asn Ala Leu Ser Leu Leu Ser Thr Phe Gly Lys Gln Asn Tyr Ser Thr
          130          135          140

gga tgc ttg tca tta tgc aat tct caa cca gag gca aat gga aga tgt     480
Gly Cys Leu Ser Leu Cys Asn Ser Gln Pro Glu Ala Asn Gly Arg Cys
          145          150          155          160

aat ggt gta ggt tgc tgc aca aca gag gac ttc tct gtc ccg ttc gat     528
Asn Gly Val Gly Cys Cys Thr Thr Glu Asp Phe Ser Val Pro Phe Asp
          165          170          175

agc gat aca ttc caa ttt ggc tca gtt cgc ttg aga aac caa gtt aat     576
Ser Asp Thr Phe Gln Phe Gly Ser Val Arg Leu Arg Asn Gln Val Asn
          180          185          190

aat tcc tta gat cta ttt aat act tcg gta tat cag ttt aat cct tgc     624
Asn Ser Leu Asp Leu Phe Asn Thr Ser Val Tyr Gln Phe Asn Pro Cys
          195          200          205

acc tac gct ttt ctc gtt gaa gat ggt aag ttt aac ttc gat tct tca     672
Thr Tyr Ala Phe Leu Val Glu Asp Gly Lys Phe Asn Phe Asp Ser Ser
          210          215          220

aaa gat ctt aag aat ctg agg aat gtc acg agg ttc cct gtg gca cta     720
Lys Asp Leu Lys Asn Leu Arg Asn Val Thr Arg Phe Pro Val Ala Leu
          225          230          235          240

gat tgg tct att gga aac cag aca tgt gag caa gct gga agc aca aga     768
Asp Trp Ser Ile Gly Asn Gln Thr Cys Glu Gln Ala Gly Ser Thr Arg
          245          250          255

ata tgc ggt aag aac agc tca tgt tac aat tct act act aga aac ggg     816
Ile Cys Gly Lys Asn Ser Ser Cys Tyr Asn Ser Thr Thr Arg Asn Gly
          260          265          270

tat atc tgc aaa tgt aat gaa ggt tat gat ggg aat cca tac cgt tca     864
Tyr Ile Cys Lys Cys Asn Glu Gly Tyr Asp Gly Asn Pro Tyr Arg Ser
          275          280          285

gag ggt tgc aaa gac atc gat gag tgt att agt gat aca cat aac tgt     912

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Ser	Asp	Pro	Lys	Thr	Cys	Arg	Asn	Arg	Asp	Gly	Gly	Phe	Asp	Cys	Lys	
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tgt	cca	tct	ggc	tac	gac	tta	aac	tcc	agt	atg	agc	tgc	acg	agg	ccc	1008
Cys	Pro	Ser	Gly	Tyr	Asp	Leu	Asn	Ser	Ser	Met	Ser	Cys	Thr	Arg	Pro	
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gaa	tac	aaa	cgg	act	cga	att	ttt	ctt	gta	atc	ata	atc	ggc	gtc	ttg	1056
Glu	Tyr	Lys	Arg	Thr	Arg	Ile	Phe	Leu	Val	Ile	Ile	Ile	Gly	Val	Leu	
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gtc	ctc	ctg	ctt	gct	gcg	atc	tgt	ata	caa	cat	gca	acg	aag	caa	agg	1104
Val	Leu	Leu	Leu	Ala	Ala	Ile	Cys	Ile	Gln	His	Ala	Thr	Lys	Gln	Arg	
				355				360					365			
aag	tat	acc	aag	ctc	cga	cga	caa	ttc	ttt	gag	caa	aat	ggc	ggc	ggc	1152
Lys	Tyr	Thr	Lys	Leu	Arg	Arg	Gln	Phe	Phe	Glu	Gln	Asn	Gly	Gly	Gly	
				370				375					380			
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Met	Leu	Ile	Gln	Arg	Leu	Ser	Gly	Ala	Gly	Leu	Ser	Asn	Ile	Asp	Phe	
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aaa	atc	ttt	act	gag	gaa	ggc	atg	aaa	gag	gca	act	aat	ggc	tat	gat	1248
Lys	Ile	Phe	Thr	Glu	Glu	Gly	Met	Lys	Glu	Ala	Thr	Asn	Gly	Tyr	Asp	
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gag	agc	aga	atc	ttg	ggc	cag	gga	ggc	caa	gga	aca	gtc	tac	aaa	ggg	1296
Glu	Ser	Arg	Ile	Leu	Gly	Gln	Gly	Gly	Gln	Gly	Thr	Val	Tyr	Lys	Gly	
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ata	ttg	ccg	gac	aac	act	atc	gtt	gct	ata	aag	aaa	gct	cgg	ctt	gca	1344
Ile	Leu	Pro	Asp	Asn	Thr	Ile	Val	Ala	Ile	Lys	Lys	Ala	Arg	Leu	Ala	
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gac	agt	aga	caa	gta	gat	cag	ttc	atc	cac	gaa	gtg	ctc	gtg	ctt	tca	1392
Asp	Ser	Arg	Gln	Val	Asp	Gln	Phe	Ile	His	Glu	Val	Leu	Val	Leu	Ser	
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caa	att	aac	cac	agg	aac	gtg	gtc	aag	atc	ttg	ggc	tgc	tgt	cta	gag	1440
Gln	Ile	Asn	His	Arg	Asn	Val	Val	Lys	Ile	Leu	Gly	Cys	Cys	Leu	Glu	
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act	gaa	gtc	ccc	ttg	ttg	gtc	tat	gaa	ttc	att	acc	aat	ggc	acc	ctt	1488
Thr	Glu	Val	Pro	Leu	Leu	Val	Tyr	Glu	Phe	Ile	Thr	Asn	Gly	Thr	Leu	
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ttc	gat	cac	ttg	cac	ggc	tcc	att	ttt	gat	tct	tct	ctt	aca	tgg	gaa	1536
Phe	Asp	His	Leu	His	Gly	Ser	Ile	Phe	Asp	Ser	Ser	Leu	Thr	Trp	Glu	
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cac	cgc	ctc	aga	ata	gcg	ata	gaa	gtc	gct	gga	act	ctt	gct	tat	ctt	1584
His	Arg	Leu	Arg	Ile	Ala	Ile	Glu	Val	Ala	Gly	Thr	Leu	Ala	Tyr	Leu	
				515				520					525			
cac	tcc	tct	gct	tct	att	cca	atc	atc	cat	cgc	gat	atc	aaa	act	gca	1632
His	Ser	Ser	Ala	Ser	Ile	Pro	Ile	Ile	His	Arg	Asp	Ile	Lys	Thr	Ala	
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aat	att	ctc	ttg	gat	gaa	aac	tta	act	gca	aaa	gta	gcc	gac	ttt	ggc	1680
Asn	Ile	Leu	Leu	Asp	Glu	Asn	Leu	Thr	Ala	Lys	Val	Ala	Asp	Phe	Gly	
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gct	tct	aag	ctt	ata	cca	atg	gat	aaa	gag	cag	ctc	aca	act	atg	gtg	1728
Ala	Ser	Lys	Leu	Ile	Pro	Met	Asp	Lys	Glu	Gln	Leu	Thr	Thr	Met	Val	
				565					570					575		
caa	ggc	act	cta	ggc	tat	tta	gac	cca	gaa	tac	tat	acc	aca	ggg	ctt	1776
Gln	Gly	Thr	Leu	Gly	Tyr	Leu	Asp	Pro	Glu	Tyr	Tyr	Thr	Thr	Gly	Leu	
				580				585					590			
ctg	aac	gag	aag	agc	gat	gtg	tat	agc	ttt	ggg	gta	gtc	ctc	atg	gaa	1824
Leu	Asn	Glu	Lys	Ser	Asp	Val	Tyr	Ser	Phe	Gly	Val	Val	Leu	Met	Glu	
				595				600					605			

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ctg ctc tca ggt caa aag gca ttg tgc ttt gaa cgg cca cag gct tca	1872
Leu Leu Ser Gly Gln Lys Ala Leu Cys Phe Glu Arg Pro Gln Ala Ser	
610 615 620	
aaa cat ttg gtg agt tac ttt gtt tct gcc acg gaa gag aat agg ttg	1920
Lys His Leu Val Ser Tyr Phe Val Ser Ala Thr Glu Glu Asn Arg Leu	
625 630 635 640	
cat gag att att gac gac caa gtg ttg aac gag gat aat ctg aag gag	1968
His Glu Ile Ile Asp Asp Gln Val Leu Asn Glu Asp Asn Leu Lys Glu	
645 650 655	
atc cag gaa gct gca aga att gct gca gag tgt aca agg cta atg gga	2016
Ile Gln Glu Ala Ala Arg Ile Ala Ala Glu Cys Thr Arg Leu Met Gly	
660 665 670	
gag gaa agg cca agg atg aaa gaa gta gct gca aag cta gaa gcc ttg	2064
Glu Glu Arg Pro Arg Met Lys Glu Val Ala Ala Lys Leu Glu Ala Leu	
675 680 685	
agg gtc gag aaa acc aaa cat aag tgg tgc gat cag tat cct gag gag	2112
Arg Val Glu Lys Thr Lys His Lys Trp Ser Asp Gln Tyr Pro Glu Glu	
690 695 700	
aat gaa cac ttg att ggt ggt cac atc ttg tct gca caa ggc gaa acc	2160
Asn Glu His Leu Ile Gly Gly His Ile Leu Ser Ala Gln Gly Glu Thr	
705 710 715 720	
agt agc agc att ggc tat gat agc atc aaa aat gta gca ata ttg gac	2208
Ser Ser Ser Ile Gly Tyr Asp Ser Ile Lys Asn Val Ala Ile Leu Asp	
725 730 735	
att gaa act ggc cgc tga	2226
Ile Glu Thr Gly Arg	
740	

<210> SEQ ID NO 8

<211> LENGTH: 741

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 8

Met Lys Phe Gln Glu Gly Val Phe Leu Val Val Ile Phe Phe Leu Ala	
1 5 10 15	
Tyr Thr Gln Leu Val Lys Gly Gln His Gln Pro Arg Glu Asp Cys Lys	
20 25 30	
Leu Lys Cys Gly Asn Val Thr Ile Glu Tyr Pro Phe Gly Ile Ser Thr	
35 40 45	
Gly Cys Tyr Tyr Pro Gly Asp Asp Asn Phe Asn Leu Thr Cys Val Val	
50 55 60	
Glu Glu Lys Leu Leu Leu Phe Gly Ile Ile Gln Val Thr Asn Ile Ser	
65 70 75 80	
His Ser Gly His Val Ser Val Leu Phe Glu Arg Phe Ser Glu Cys Tyr	
85 90 95	
Glu Gln Lys Asn Glu Thr Asn Gly Thr Ala Leu Gly Tyr Gln Leu Gly	
100 105 110	
Ser Ser Phe Ser Leu Ser Ser Asn Asn Lys Phe Thr Leu Val Gly Cys	
115 120 125	
Asn Ala Leu Ser Leu Leu Ser Thr Phe Gly Lys Gln Asn Tyr Ser Thr	
130 135 140	
Gly Cys Leu Ser Leu Cys Asn Ser Gln Pro Glu Ala Asn Gly Arg Cys	
145 150 155 160	
Asn Gly Val Gly Cys Cys Thr Thr Glu Asp Phe Ser Val Pro Phe Asp	
165 170 175	
Ser Asp Thr Phe Gln Phe Gly Ser Val Arg Leu Arg Asn Gln Val Asn	
180 185 190	

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Asn	Ser	Leu	Asp	Leu	Phe	Asn	Thr	Ser	Val	Tyr	Gln	Phe	Asn	Pro	Cys
	195						200					205			
Thr	Tyr	Ala	Phe	Leu	Val	Glu	Asp	Gly	Lys	Phe	Asn	Phe	Asp	Ser	Ser
	210						215				220				
Lys	Asp	Leu	Lys	Asn	Leu	Arg	Asn	Val	Thr	Arg	Phe	Pro	Val	Ala	Leu
	225				230					235					240
Asp	Trp	Ser	Ile	Gly	Asn	Gln	Thr	Cys	Glu	Gln	Ala	Gly	Ser	Thr	Arg
			245						250					255	
Ile	Cys	Gly	Lys	Asn	Ser	Ser	Cys	Tyr	Asn	Ser	Thr	Thr	Arg	Asn	Gly
		260						265					270		
Tyr	Ile	Cys	Lys	Cys	Asn	Glu	Gly	Tyr	Asp	Gly	Asn	Pro	Tyr	Arg	Ser
		275					280					285			
Glu	Gly	Cys	Lys	Asp	Ile	Asp	Glu	Cys	Ile	Ser	Asp	Thr	His	Asn	Cys
	290					295					300				
Ser	Asp	Pro	Lys	Thr	Cys	Arg	Asn	Arg	Asp	Gly	Gly	Phe	Asp	Cys	Lys
	305				310					315					320
Cys	Pro	Ser	Gly	Tyr	Asp	Leu	Asn	Ser	Ser	Met	Ser	Cys	Thr	Arg	Pro
			325						330					335	
Glu	Tyr	Lys	Arg	Thr	Arg	Ile	Phe	Leu	Val	Ile	Ile	Ile	Gly	Val	Leu
		340						345					350		
Val	Leu	Leu	Leu	Ala	Ala	Ile	Cys	Ile	Gln	His	Ala	Thr	Lys	Gln	Arg
	355						360					365			
Lys	Tyr	Thr	Lys	Leu	Arg	Arg	Gln	Phe	Phe	Glu	Gln	Asn	Gly	Gly	Gly
	370					375					380				
Met	Leu	Ile	Gln	Arg	Leu	Ser	Gly	Ala	Gly	Leu	Ser	Asn	Ile	Asp	Phe
	385				390					395					400
Lys	Ile	Phe	Thr	Glu	Glu	Gly	Met	Lys	Glu	Ala	Thr	Asn	Gly	Tyr	Asp
			405					410						415	
Glu	Ser	Arg	Ile	Leu	Gly	Gln	Gly	Gly	Gln	Gly	Thr	Val	Tyr	Lys	Gly
		420					425						430		
Ile	Leu	Pro	Asp	Asn	Thr	Ile	Val	Ala	Ile	Lys	Lys	Ala	Arg	Leu	Ala
	435						440					445			
Asp	Ser	Arg	Gln	Val	Asp	Gln	Phe	Ile	His	Glu	Val	Leu	Val	Leu	Ser
	450					455					460				
Gln	Ile	Asn	His	Arg	Asn	Val	Val	Lys	Ile	Leu	Gly	Cys	Cys	Leu	Glu
	465				470					475					480
Thr	Glu	Val	Pro	Leu	Leu	Val	Tyr	Glu	Phe	Ile	Thr	Asn	Gly	Thr	Leu
			485					490						495	
Phe	Asp	His	Leu	His	Gly	Ser	Ile	Phe	Asp	Ser	Ser	Leu	Thr	Trp	Glu
		500						505					510		
His	Arg	Leu	Arg	Ile	Ala	Ile	Glu	Val	Ala	Gly	Thr	Leu	Ala	Tyr	Leu
	515						520					525			
His	Ser	Ser	Ala	Ser	Ile	Pro	Ile	Ile	His	Arg	Asp	Ile	Lys	Thr	Ala
	530					535					540				
Asn	Ile	Leu	Leu	Asp	Glu	Asn	Leu	Thr	Ala	Lys	Val	Ala	Asp	Phe	Gly
	545				550					555					560
Ala	Ser	Lys	Leu	Ile	Pro	Met	Asp	Lys	Glu	Gln	Leu	Thr	Thr	Met	Val
			565					570						575	
Gln	Gly	Thr	Leu	Gly	Tyr	Leu	Asp	Pro	Glu	Tyr	Tyr	Thr	Thr	Gly	Leu
		580					585						590		
Leu	Asn	Glu	Lys	Ser	Asp	Val	Tyr	Ser	Phe	Gly	Val	Val	Leu	Met	Glu
	595						600					605			

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Leu Leu Ser Gly Gln Lys Ala Leu Cys Phe Glu Arg Pro Gln Ala Ser
 610 615 620
 Lys His Leu Val Ser Tyr Phe Val Ser Ala Thr Glu Glu Asn Arg Leu
 625 630 635 640
 His Glu Ile Ile Asp Asp Gln Val Leu Asn Glu Asp Asn Leu Lys Glu
 645 650 655
 Ile Gln Glu Ala Ala Arg Ile Ala Ala Glu Cys Thr Arg Leu Met Gly
 660 665 670
 Glu Glu Arg Pro Arg Met Lys Glu Val Ala Ala Lys Leu Glu Ala Leu
 675 680 685
 Arg Val Glu Lys Thr Lys His Lys Trp Ser Asp Gln Tyr Pro Glu Glu
 690 695 700
 Asn Glu His Leu Ile Gly Gly His Ile Leu Ser Ala Gln Gly Glu Thr
 705 710 715 720
 Ser Ser Ser Ile Gly Tyr Asp Ser Ile Lys Asn Val Ala Ile Leu Asp
 725 730 735
 Ile Glu Thr Gly Arg
 740

<210> SEQ ID NO 9

<211> LENGTH: 2202

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(2199)

<223> OTHER INFORMATION: Wall-associated kinase 5, cDNA, complete CDS

<400> SEQUENCE: 9

atg aag gtg cat agt ctg ttc ttg atg gct att ttc ttc tac cta gca	48
Met Lys Val His Ser Leu Phe Leu Met Ala Ile Phe Phe Tyr Leu Ala	
1 5 10 15	
tat acg cag ctg gtc aag gcg caa cct cgc gat gat tgc caa act aga	96
Tyr Thr Gln Leu Val Lys Ala Gln Pro Arg Asp Asp Cys Gln Thr Arg	
20 25 30	
tgt ggt gac gtc cca att gat tac cct ttt ggt att tct aca ggt tgt	144
Cys Gly Asp Val Pro Ile Asp Tyr Pro Phe Gly Ile Ser Thr Gly Cys	
35 40 45	
tac tac ccc gga gat gat agc ttc aat att acc tgt gag gaa gat aaa	192
Tyr Tyr Pro Gly Asp Asp Ser Phe Asn Ile Thr Cys Glu Glu Asp Lys	
50 55 60	
cca aat gtc tta agc aac att gaa gtg cta aac ttt aat cat agc ggc	240
Pro Asn Val Leu Ser Asn Ile Glu Val Leu Asn Phe Asn His Ser Gly	
65 70 75 80	
cag cta cgc ggt ctg att cct cga tcc act gtt tgc tac gac cag caa	288
Gln Leu Arg Gly Leu Ile Pro Arg Ser Thr Val Cys Tyr Asp Gln Gln	
85 90 95	
aca aat aat gat ttc gag tcc ctc tgg ttt cgg ttg gat aat tta tct	336
Thr Asn Asn Asp Phe Glu Ser Leu Trp Phe Arg Leu Asp Asn Leu Ser	
100 105 110	
ttc tcc ccc aat aac aag ttt act tta gta ggc tgt aac gct tgg gca	384
Phe Ser Pro Asn Asn Lys Phe Thr Leu Val Gly Cys Asn Ala Trp Ala	
115 120 125	
ctt cta agc act ttt gga ata caa aac tac tca act gga tgt atg tca	432
Leu Leu Ser Thr Phe Gly Ile Gln Asn Tyr Ser Thr Gly Cys Met Ser	
130 135 140	
tta tgc gat act ccc ccg ccg cca aat agt aaa tgt aat ggt gtt ggt	480
Leu Cys Asp Thr Pro Pro Pro Asn Ser Lys Cys Asn Gly Val Gly	
145 150 155 160	

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tgc tgc aga aca gag gta tct atc ccc ttg gat agc cat aga att gaa	528
Cys Cys Arg Thr Glu Val Ser Ile Pro Leu Asp Ser His Arg Ile Glu	
165 170 175	
act caa cca tct cgc ttc gaa aac atg act tcc gtg gag cac ttt aat	576
Thr Gln Pro Ser Arg Phe Glu Asn Met Thr Ser Val Glu His Phe Asn	
180 185 190	
cct tgc agc tac gct ttt ttc gtt gaa gat ggt atg ttt aac ttc agt	624
Pro Cys Ser Tyr Ala Phe Phe Val Glu Asp Gly Met Phe Asn Phe Ser	
195 200 205	
tct tta gaa gat ctt aag gat ctg cga aat gtc acg agg ttc cct gtg	672
Ser Leu Glu Asp Leu Lys Asp Leu Arg Asn Val Thr Arg Phe Pro Val	
210 215 220	
tta cta gat tgg tct att gga aac cag aca tgt gag caa gtt gta ggt	720
Leu Leu Asp Trp Ser Ile Gly Asn Gln Thr Cys Glu Gln Val Val Gly	
225 230 235 240	
aga aac ata tgt ggt ggg aac agc aca tgt ttt gat tct act cgt gga	768
Arg Asn Ile Cys Gly Gly Asn Ser Thr Cys Phe Asp Ser Thr Arg Gly	
245 250 255	
aag ggt tat aac tgc aag tgt tta caa ggt ttt gat ggg aat cca tac	816
Lys Gly Tyr Asn Cys Lys Cys Leu Gln Gly Phe Asp Gly Asn Pro Tyr	
260 265 270	
ctt tcg gac ggt tgc caa gac atc aat gag tgt act acc cgt ata cat	864
Leu Ser Asp Gly Cys Gln Asp Ile Asn Glu Cys Thr Thr Arg Ile His	
275 280 285	
aac tgt tcg gat acc agc aca tgt gaa aac aca ctt gga agc ttc cat	912
Asn Cys Ser Asp Thr Ser Thr Cys Glu Asn Thr Leu Gly Ser Phe His	
290 295 300	
tgt cag tgc cca tct ggt tct gat tta aat acc act act atg agc tgc	960
Cys Gln Cys Pro Ser Gly Ser Asp Leu Asn Thr Thr Thr Met Ser Cys	
305 310 315 320	
att gac aca cct aaa gaa gag cct aag tac tta gga tgg act act gtt	1008
Ile Asp Thr Pro Lys Glu Glu Pro Lys Tyr Leu Gly Trp Thr Thr Val	
325 330 335	
ctt ctt gga acc acc atc gga ttc tta atc atc ttg ctt acc att agc	1056
Leu Leu Gly Thr Thr Ile Gly Phe Leu Ile Ile Leu Leu Thr Ile Ser	
340 345 350	
tat ata caa caa aaa atg agg cac cga aaa aac acc gag ctg cga caa	1104
Tyr Ile Gln Gln Lys Met Arg His Arg Lys Asn Thr Glu Leu Arg Gln	
355 360 365	
caa ttc ttc gag caa aat ggt ggt ggc atg ttg ata cag cga ctc tca	1152
Gln Phe Phe Glu Gln Asn Gly Gly Gly Met Leu Ile Gln Arg Leu Ser	
370 375 380	
gga gca ggg cca tca aat gtg gat gtc aaa atc ttt act gaa gaa ggc	1200
Gly Ala Gly Pro Ser Asn Val Asp Val Lys Ile Phe Thr Glu Glu Gly	
385 390 395 400	
atg aag gaa gca act gat ggt tat aat gag agc aga atc cta ggc cag	1248
Met Lys Glu Ala Thr Asp Gly Tyr Asn Glu Ser Arg Ile Leu Gly Gln	
405 410 415	
gga gga caa gga aca gtc tac aaa ggg ata ttg caa gat aac tcc att	1296
Gly Gly Gln Gly Thr Val Tyr Lys Gly Ile Leu Gln Asp Asn Ser Ile	
420 425 430	
gtt gct ata aag aaa gct cga ctt gga gac cgt agc caa gta gag cag	1344
Val Ala Ile Lys Lys Ala Arg Leu Gly Asp Arg Ser Gln Val Glu Gln	
435 440 445	
ttc atc aac gaa gtg cta gtg ctt tca caa ata aac cac agg aac gtg	1392
Phe Ile Asn Glu Val Leu Val Leu Ser Gln Ile Asn His Arg Asn Val	
450 455 460	
gtc aaa ctc ttg ggc tgt tgt cta gag act gaa gtt ccc ttg ttg gtc	1440
Val Lys Leu Leu Gly Cys Cys Leu Glu Thr Glu Val Pro Leu Leu Val	
465 470 475 480	

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tat gag ttc att tcc agt ggc act ctt ttt gat cac ttg cac ggt tct Tyr Glu Phe Ile Ser Ser Gly Thr Leu Phe Asp His Leu His Gly Ser 485 490 495	1488
atg ttt gat tct tcg ctt aca tgg gaa cac cgt ctg agg ata gcc ata Met Phe Asp Ser Ser Leu Thr Trp Glu His Arg Leu Arg Ile Ala Ile 500 505 510	1536
gaa gtt gct gga act ctt gca tat ctt cac tcc tat gct tct att cca Glu Val Ala Gly Thr Leu Ala Tyr Leu His Ser Tyr Ala Ser Ile Pro 515 520 525	1584
atc atc cac cga gat gtc aag act gct aac att ctc ctc gat gaa aac Ile Ile His Arg Asp Val Lys Thr Ala Asn Ile Leu Leu Asp Glu Asn 530 535 540	1632
tta act gca aaa gta gct gat ttt ggt gca tca agg ctg ata ccg atg Leu Thr Ala Lys Val Ala Asp Phe Gly Ala Ser Arg Leu Ile Pro Met 545 550 555 560	1680
gac caa gag cag ctc aca act atg gtt caa gga act ctt ggc tat tta Asp Gln Glu Gln Leu Thr Thr Met Val Gln Gly Thr Leu Gly Tyr Leu 565 570 575	1728
gac cct gaa tac tac aat aca ggg ctt ctg aac gaa aag agc gat gtt Asp Pro Glu Tyr Tyr Asn Thr Gly Leu Leu Asn Glu Lys Ser Asp Val 580 585 590	1776
tat agc ttt ggg gta gtc ctc atg gaa ctg ctc tca ggt gaa aag gca Tyr Ser Phe Gly Val Val Leu Met Glu Leu Leu Ser Gly Glu Lys Ala 595 600 605	1824
tta tgc ttt gaa cgg cca caa agc tca aaa cat cta gtg agt tac ttt Leu Cys Phe Glu Arg Pro Gln Ser Ser Lys His Leu Val Ser Tyr Phe 610 615 620	1872
gtt tct gcc atg aaa gaa aat agg ttg cat gag att att gac ggt caa Val Ser Ala Met Lys Glu Asn Arg Leu His Glu Ile Ile Asp Gly Gln 625 630 635 640	1920
gtt atg aac gag tat aat cag agg gag atc cag gaa tct gca aga att Val Met Asn Glu Tyr Asn Gln Arg Glu Ile Gln Glu Ser Ala Arg Ile 645 650 655	1968
gct gtt gag tgt aca aga att atg gga gag gaa agg cca agt atg aaa Ala Val Glu Cys Thr Arg Ile Met Gly Glu Glu Arg Pro Ser Met Lys 660 665 670	2016
gaa gta gct gca gag tta gag gcc ttg aga gtc aaa aca acc aaa cat Glu Val Ala Ala Glu Leu Glu Ala Leu Arg Val Lys Thr Thr Lys His 675 680 685	2064
cag tgg tca gat caa tat ccc aag gag gtt gag cat ttg ctt ggt gtt Gln Trp Ser Asp Gln Tyr Pro Lys Glu Val Glu His Leu Leu Gly Val 690 695 700	2112
caa atc tta tcg acg caa ggt gat acc agt agc att ggc tat gac agc Gln Ile Leu Ser Thr Gln Gly Asp Thr Ser Ser Ile Gly Tyr Asp Ser 705 710 715 720	2160
atc cag aat gta aca agg ttg gac att gaa act ggc cgc tga Ile Gln Asn Val Thr Arg Leu Asp Ile Glu Thr Gly Arg 725 730	2202

<210> SEQ ID NO 10

<211> LENGTH: 733

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 10

Met Lys Val His Ser Leu Phe Leu Met Ala Ile Phe Phe Tyr Leu Ala
1 5 10 15

Tyr Thr Gln Leu Val Lys Ala Gln Pro Arg Asp Asp Cys Gln Thr Arg
20 25 30

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Cys	Gly	Asp	Val	Pro	Ile	Asp	Tyr	Pro	Phe	Gly	Ile	Ser	Thr	Gly	Cys
		35					40					45			
Tyr	Tyr	Pro	Gly	Asp	Asp	Ser	Phe	Asn	Ile	Thr	Cys	Glu	Glu	Asp	Lys
	50					55					60				
Pro	Asn	Val	Leu	Ser	Asn	Ile	Glu	Val	Leu	Asn	Phe	Asn	His	Ser	Gly
65					70					75					80
Gln	Leu	Arg	Gly	Leu	Ile	Pro	Arg	Ser	Thr	Val	Cys	Tyr	Asp	Gln	Gln
			85						90					95	
Thr	Asn	Asn	Asp	Phe	Glu	Ser	Leu	Trp	Phe	Arg	Leu	Asp	Asn	Leu	Ser
			100					105					110		
Phe	Ser	Pro	Asn	Asn	Lys	Phe	Thr	Leu	Val	Gly	Cys	Asn	Ala	Trp	Ala
			115				120					125			
Leu	Leu	Ser	Thr	Phe	Gly	Ile	Gln	Asn	Tyr	Ser	Thr	Gly	Cys	Met	Ser
	130					135					140				
Leu	Cys	Asp	Thr	Pro	Pro	Pro	Pro	Asn	Ser	Lys	Cys	Asn	Gly	Val	Gly
145					150					155					160
Cys	Cys	Arg	Thr	Glu	Val	Ser	Ile	Pro	Leu	Asp	Ser	His	Arg	Ile	Glu
				165					170					175	
Thr	Gln	Pro	Ser	Arg	Phe	Glu	Asn	Met	Thr	Ser	Val	Glu	His	Phe	Asn
			180					185					190		
Pro	Cys	Ser	Tyr	Ala	Phe	Phe	Val	Glu	Asp	Gly	Met	Phe	Asn	Phe	Ser
		195					200					205			
Ser	Leu	Glu	Asp	Leu	Lys	Asp	Leu	Arg	Asn	Val	Thr	Arg	Phe	Pro	Val
	210					215					220				
Leu	Leu	Asp	Trp	Ser	Ile	Gly	Asn	Gln	Thr	Cys	Glu	Gln	Val	Val	Gly
225					230					235					240
Arg	Asn	Ile	Cys	Gly	Gly	Asn	Ser	Thr	Cys	Phe	Asp	Ser	Thr	Arg	Gly
			245						250					255	
Lys	Gly	Tyr	Asn	Cys	Lys	Cys	Leu	Gln	Gly	Phe	Asp	Gly	Asn	Pro	Tyr
		260						265					270		
Leu	Ser	Asp	Gly	Cys	Gln	Asp	Ile	Asn	Glu	Cys	Thr	Thr	Arg	Ile	His
		275					280					285			
Asn	Cys	Ser	Asp	Thr	Ser	Thr	Cys	Glu	Asn	Thr	Leu	Gly	Ser	Phe	His
	290					295					300				
Cys	Gln	Cys	Pro	Ser	Gly	Ser	Asp	Leu	Asn	Thr	Thr	Thr	Met	Ser	Cys
305					310					315					320
Ile	Asp	Thr	Pro	Lys	Glu	Glu	Pro	Lys	Tyr	Leu	Gly	Trp	Thr	Thr	Val
			325						330					335	
Leu	Leu	Gly	Thr	Thr	Ile	Gly	Phe	Leu	Ile	Ile	Leu	Leu	Thr	Ile	Ser
			340					345					350		
Tyr	Ile	Gln	Gln	Lys	Met	Arg	His	Arg	Lys	Asn	Thr	Glu	Leu	Arg	Gln
		355					360					365			
Gln	Phe	Phe	Glu	Gln	Asn	Gly	Gly	Gly	Met	Leu	Ile	Gln	Arg	Leu	Ser
	370					375					380				
Gly	Ala	Gly	Pro	Ser	Asn	Val	Asp	Val	Lys	Ile	Phe	Thr	Glu	Glu	Gly
385					390					395					400
Met	Lys	Glu	Ala	Thr	Asp	Gly	Tyr	Asn	Glu	Ser	Arg	Ile	Leu	Gly	Gln
			405						410					415	
Gly	Gly	Gln	Gly	Thr	Val	Tyr	Lys	Gly	Ile	Leu	Gln	Asp	Asn	Ser	Ile
			420					425					430		
Val	Ala	Ile	Lys	Lys	Ala	Arg	Leu	Gly	Asp	Arg	Ser	Gln	Val	Glu	Gln
		435					440					445			
Phe	Ile	Asn	Glu	Val	Leu	Val	Leu	Ser	Gln	Ile	Asn	His	Arg	Asn	Val

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450	455	460
Val Lys Leu Leu Gly Cys Cys Leu Glu Thr Glu Val Pro Leu Leu Val		
465	470	475 480
Tyr Glu Phe Ile Ser Ser Gly Thr Leu Phe Asp His Leu His Gly Ser		
	485	490 495
Met Phe Asp Ser Ser Leu Thr Trp Glu His Arg Leu Arg Ile Ala Ile		
	500	505 510
Glu Val Ala Gly Thr Leu Ala Tyr Leu His Ser Tyr Ala Ser Ile Pro		
	515	520 525
Ile Ile His Arg Asp Val Lys Thr Ala Asn Ile Leu Leu Asp Glu Asn		
	530	535 540
Leu Thr Ala Lys Val Ala Asp Phe Gly Ala Ser Arg Leu Ile Pro Met		
545	550	555 560
Asp Gln Glu Gln Leu Thr Thr Met Val Gln Gly Thr Leu Gly Tyr Leu		
	565	570 575
Asp Pro Glu Tyr Tyr Asn Thr Gly Leu Leu Asn Glu Lys Ser Asp Val		
	580	585 590
Tyr Ser Phe Gly Val Val Leu Met Glu Leu Leu Ser Gly Glu Lys Ala		
	595	600 605
Leu Cys Phe Glu Arg Pro Gln Ser Ser Lys His Leu Val Ser Tyr Phe		
	610	615 620
Val Ser Ala Met Lys Glu Asn Arg Leu His Glu Ile Ile Asp Gly Gln		
625	630	635 640
Val Met Asn Glu Tyr Asn Gln Arg Glu Ile Gln Glu Ser Ala Arg Ile		
	645	650 655
Ala Val Glu Cys Thr Arg Ile Met Gly Glu Glu Arg Pro Ser Met Lys		
	660	665 670
Glu Val Ala Ala Glu Leu Glu Ala Leu Arg Val Lys Thr Thr Lys His		
	675	680 685
Gln Trp Ser Asp Gln Tyr Pro Lys Glu Val Glu His Leu Leu Gly Val		
	690	695 700
Gln Ile Leu Ser Thr Gln Gly Asp Thr Ser Ser Ile Gly Tyr Asp Ser		
705	710	715 720
Ile Gln Asn Val Thr Arg Leu Asp Ile Glu Thr Gly Arg		
	725	730

<210> SEQ ID NO 11

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
WAK_NDE primer

<400> SEQUENCE: 11

catatgaaag tgcagcgctct gtt

23

<210> SEQ ID NO 12

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
WAK_XBA primer

<400> SEQUENCE: 12

tctagatcag cggcctgctt caa

23

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What is claimed is:

1. A nucleic acid construct for increasing fiber length and/or plant height, said construct comprising a wall-associated kinase 4 (WAK4) polynucleotide sequence operably linked to a xylem-preferred promoter that causes overexpression of said WAK4 polynucleotide sequence, wherein said WAK4 polynucleotide sequence encodes a polypeptide having at least 95% or more amino acid sequence identity to SEQ ID NO: 2.
2. The nucleic acid construct of claim 1, wherein said xylem-preferred promoter is selected from the group consisting of TUB gene promoter, SuSy gene promoter, COMT gene promoter and C4H gene promoter.
3. A transgenic plant comprising a nucleic acid construct comprising a WAK4 polynucleotide sequence operably linked to a xylem-preferred promoter that causes overexpression of said WAK4 polynucleotide sequence, wherein said WAK4 polynucleotide sequence encodes a polypeptide having at least 95% or more amino acid sequence identity to SEQ ID NO: 2, wherein said plant has an increase in fiber length and/or plant height compared to a non-transgenic plant of the same species.
4. The transgenic plant of claim 3, wherein the xylem-preferred promoter is selected from the group consisting of TUB gene promoter, SuSy gene promoter, COMT gene promoter, and C4H gene promoter.
5. The transgenic plant of claim 3, wherein said plant is a dicotyledon plant.
6. The transgenic plant of claim 3, wherein said plant is a monocotyledon plant.
7. The transgenic plant of claim 3, wherein said plant is a gymnosperm.
8. The transgenic plant of claim 3, wherein said plant is a hardwood tree.
9. The transgenic plant of claim 8, wherein said hardwood tree is an *Eucalyptus* tree.
10. The transgenic plant of claim 8, wherein said hardwood tree is a *Populus* tree.
11. The transgenic plant of claim 7, wherein said gymnosperm is a *Pinus* tree.
12. A part of the transgenic plant of claim 3, wherein said part is selected from the group consisting of a leaf, a stem, a

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flower, an ovary, a fruit, a seed, and a callus, and wherein said part comprises said nucleic acid construct.

13. A progeny of the transgenic plant of claim 3, wherein said progeny comprises said nucleic acid construct.

14. The progeny of claim 13, wherein said progeny is a hybrid plant and wherein said hybrid plant comprises said nucleic acid construct.

15. A method for increasing fiber length and/or plant height, comprising:

- (a) introducing into a plant cell a nucleic acid construct comprising a WAK4 polynucleotide sequence operably linked to a xylem-preferred promoter that causes overexpression of said WAK4 polynucleotide sequence, wherein said WAK4 polynucleotide sequence encodes a polypeptide having at least 95% or more amino acid sequence identity to SEQ ID NO: 2;
- (b) culturing said plant cell under conditions that promote growth of a plant; and
- (c) selecting a transgenic plant that has increased fiber length and/or plant height compared to a non-transgenic plant of the same species.

16. The method of claim 15, wherein said xylem-preferred promoter is selected from the group consisting of TUB gene promoter, SuSy gene promoter, COMT gene promoter, and C4H gene promoter.

17. A wood pulp composition comprising a WAK4 polynucleotide sequence operably linked to a xylem-preferred promoter that causes overexpression of said WAK4 polynucleotide sequence, wherein said WAK4 polynucleotide sequence encodes a polypeptide having at least 95% or more amino acid sequence identity to SEQ ID NO: 2.

18. A wood fiber composition comprising a WAK4 polynucleotide sequence operably linked to a xylem-preferred promoter that causes overexpression of said WAK4 polynucleotide sequence, wherein said WAK4 polynucleotide sequence encodes a polypeptide having at least 95% or more amino acid sequence identity to SEQ ID NO: 2.

19. The transgenic plant of claim 3, wherein said WAK4 polynucleotide sequence encodes the polypeptide of SEQ ID NO:2.

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